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BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

TRANSLATED FROM RUSSIAN

CONSULTANTS BUREAU

INTRAARTERIAL INFUSION OF PROCAINE

IN THERAPEUTIC PRACTICE

by N. K. Gorbadei

Translated from Russian

This English translation of Dr. Gorbadei's book includes as an appendix a report by I. I. Velikanov on treatment of hypertensive patients with intraarterial procaine infusions. Dr. Velikanov's report was first published in the Bulletin of the Academy of Sciences of the Kazak SSR.

Cases of peptic ulcer dating back to the siege of Leningrac responded to a series of infusions of procaine into the arteries so that the patients were able to resume their normal activities according to the author, Dr. N. K. Gorbadei who has reported on 177 cases of peptic ulcer treated at the Leningrad Sanitary Hygiene Medical Institute.

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Intravascular Infusion of Procaine in Therapeutic Practice (a survey c the literature) (A Short Account of the Properties of Procaine Intravenous Procaine Infusions; Intraarterial Procaine Infusions.)

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The Technique of Intraarterial Infusion of Procaine

The Treatment of Peptic Ulcerations by Intraarterial Procaine Infusions [Clinical Features of the Patients Under Investigation; Influence on Subjective Symptoms; Roentgenological Findings During Treatment; Acid-secreting Function of the Stomach During Treatment; Changes in the Blood Picture; Changes in the Zones of Cutaneous Sensation; State of the Cardiovascular System (Electrocardiography and Plethysmography) During Treatment.]

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The Treatment of Patients with Hypertension by Intraarterial Infusion of Procaine Solution. I. I. Velikanov

135 pages **$7.50**

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BULLETIN OF

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The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

SOME REFLEXES OF A TRANSPLANTED REINNERVATED KIDNEY

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Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50, No. 12,
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We have shown previously [3] that the interoceptors of a transplanted reinnervated kidney may be stimulated by injecting solutions of potassium, sodium, calcium,or magnesium chloride into the renal pelvis, or alternatively by injecting sodium or potassium chloride solutions into the artery supplying the kidney in its transplanted position. In the experiments described above, the solutions were injected into the kidney itself; it was then not clear whether stimulation of the interoceptors could occur through the action of substances in concentrations which might be reached in the blood under conditions of high salt intake. It was decided to investigate whether or not such an effect could occur when the concentration of the substances mentioned in the blood was high, and when the kidneys were rapidly excreting the salts. The first observations were made by G. M. Shpuga [4]. A solution of 10-15 g of sodium chloride in 700-800 ml of water was introduced into the stomach of a dog with a transplanted reinnervated kidney. In addition to showing an increased diuresis, the animal coughed and made vomiting movements without actually bringing up the solution. The transplanted kidney was reinnervated from the central end of the vagus, and the results were explained as follows: The excretion of large amounts of sodium chloride results in stimulation of the interoceptors of the transplanted kidney, which in turn leads to the typical vagal response of coughing and vomiting. A similar experiment was carried out by V. N. Chernigovskii and his co-workers [1]. They observed expectorating and vomiting movements when the animal was given a fluid load of water and milk.

The object of the present investigation has been to study this reaction in dogs, and to determine the conditions under which it arises.

METHODS

The experiments were carried out on two dogs—Zhulik (a male weighing 14.2 kg) and Daisy (a female weighing 24 kg); the right kidney was transplanted into the neck, and reinnervated by joining the central end of the vagus to the stump of the divided renal nerve. The orifice of the ureter of the intact kidney was brought out to the skin of the abdomen. At first we repeated G. M. Shpuga's experiment on a large number of dogs. However,no result was obtained from introducing salt solution into the stomach , i.e., there were neither coughing nor vomiting movements, and only in one dog was there a slight and very occasional indication of a cough. In considering this result it has occurred to us that the innervation may be established differently in each separate case, and the number of afferent fibers growing into the transplanted kidney might be different in different animals. Apparently, in our experiments the threshold of stimulation of the interoceptors of the transplanted reinnervated kidneys was too high to enable G. M. Shpuga's experiment to succeed. We therefore changed the method of introducing sodium chloride, in order to establish a higher concentration in the blood, and to cause it to be maximally excreted with the urine.

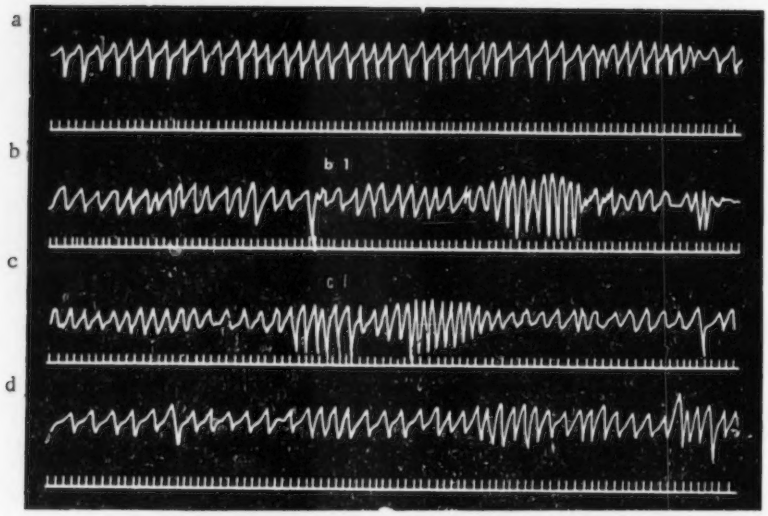

Fig. 1. Changes in respiration in the dog Zhulik following intravenous infusion of a 5% sodium chloride solution. Experiment of July 8, 1958. Duration of infusion 75 min. a) Control recording; b) 60 min after starting the infusion; c) 15 min, and d) 30 min after completing the infusion. b1) Cough; c1) cough.

It was decided to introduce sodium fluoride solution intravenously. The method has been used by other authors to study renal function [5]. In our experiments, 50-70 ml of tap water was introduced into the stomach through a catheter, in order to produce a sufficient diuresis. After 45-60 min, continuous intravenous infusion of a 5% sodium chloride solution was begun. The solution was introduced into a vein of the hind,lower leg at 4-5 ml/min for 60-90 min (usually for 75 minutes). During this time, from 280 to 400 ml of the solution was injected. The amount and the rate caused no ill effects, and no case of poisoning or death occurred. We introduced on average 18.8 ml (from 14.1 to 24.6 ml) of solution per kg weight of the animal, whereas Selkurt and Post used on average 43 ml (from 30 to 54 ml) of the same solution intravenously per kg in a shorter time.

Before the beginning of the infusion and for 1-2 hr after it, breathing movements were recorded on a smoked drum using a pneumatic transmission system. During the whole of the experiment urine was collected at 15 min intervals, and its specific gravity was measured.

After the change in respiration following infusion of 5% sodium chloride solution had been determined, the vagus branch supplying the kidney was sectioned, and the experiment again repeated. In Daisy, three experiments were carried out before section of the vagus and three after, and in Zhulik— four before and two after.

RESULTS

The experiments were begun 5-6 months after transplanting the kidney and simultaneously restoring its innervation. It was found that a light massage of the transplanted kidney caused coughing, which demonstrated that the afferent innervation had become established. The cough appeared on the 17th day after transplanting the kidney in Daisy and on the 28th day in Zhulik. By the 5-6th month, therefore, we could assume that the nerve fibers had completely regenerated.

Figure 1 shows the respiratory changes following intravenous infusion of sodium chloride. As a rule, during the first 15-20 min after the start of the infusion no changes were observed. After 30-60 min coughing started, either as a single cough, or more frequently as a series of coughing movements following closely on each other (coughing attack). In many experiments a cough occurred 15-45 min before the end of the intravenous infusion. During each experiment, the cough might occur once, though in some there were three to five coughing attacks. Out of the seven experiments on the two dogs, coughing occurred in six. In two,vomiting began 60 and 75 min after the infusion had been completed.

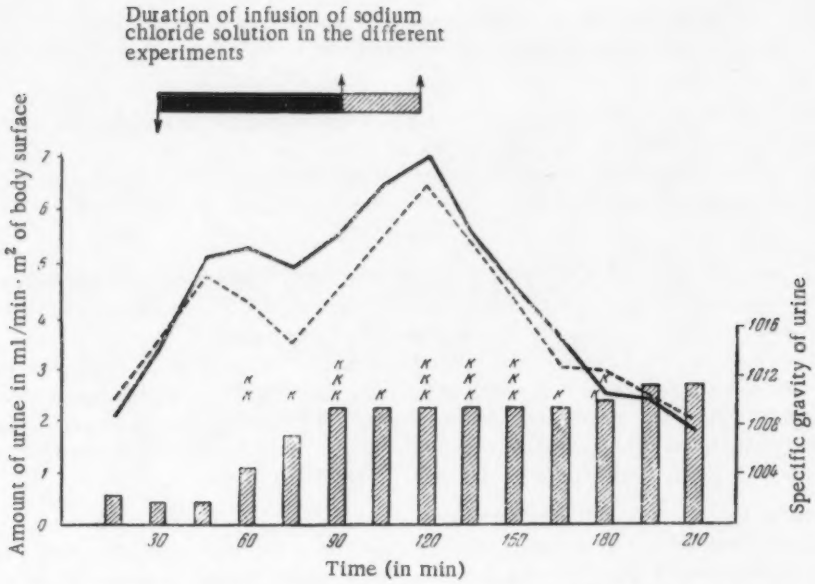

Fig. 2. Change in diuresis and the specific gravity of the urine from the reinnervated transplanted and from the unoperated kidney during intravenous infusion of a 5% sodium chloride solution (average figures for seven experiments on dogs Zhulik and Daisy).

Similar experiments were carried out on both dogs after section of the vagus supplying the transplanted kidney. Infusion of the same amount of sodium chloride at the same rate as before caused no coughing or vomiting in any of the five experiments.

In neither set of experiments was there any definite change in respiration. In Zhulik there was no change either before or after section of the vagus. In Daisy, in both experiments there was a slight increase in rate in response to the intravenous infusion of sodium chloride. Evidently the increase in respiration rate observed in certain experiments does not depend on the presence of or absence of a vagal innervation of the kidney, but is probably due to the resorptive effect of sodium chloride.

The experiments show therefore that injecting large amounts of sodium chloride into the blood stimulates the interoceptors of a transplanted reinnervated kidney, so that the typical vagal response of coughing and vomiting occurs. The correctness of this interpretation is confirmed firstly by the control experiments in which the vagus supplying the transplanted kidney was sectioned, and secondly, by experiments carried out previously [3] in which sodium chloride solution injected into the artery supplying the transplanted kidney was shown to induce a coughing attack.

In our experiments, at the same time that the respiratory movements were recorded we also measured diuresis and the specific gravity of the urine from both kidneys, and it was interesting to compare the two figures. Figure 2 shows the average results of seven experiments carried out on both dogs before vagal section. In order to compare the experiments on the two dogs, the diuresis has been referred to 1 m^2 of body surface, and the average results of seven experiments given. The specific gravity of the urine was the same in all samples for both the reinnnervated transplanted and the intact kidney, so that the results for both kidneys are given together.

After introducing water into the stomach, diuresis was noticeably increased. In the control part of the experiment, before the intravenous infusion of sodium chloride solution, the kidneys excreted about the same amounts of urine of a very low specific gravity (1.001-1.003). After the sodium chloride infusion had begun, diuresis was somewhat reduced, and then again increased. Despite the increase, the specific gravity rose to 1.010-1.014, showing that large amounts of sodium chloride were being excreted with the urine. During osmotic diuresis induced by the injection of sodium chloride, the reinnervated transplanted kidney excreted less urine than did the other, whereas for the water diuresis both kidneys produced about the same amount of urine. The difference

was not due to any incompleteness of the innervation, because the same difference remained after vagal section. It appeared that as far as osmotic diuresis was concerned, the transplanted kidney was less efficient than the intact one.

Figure 2 also shows the relationship of the time of appearance of the cough to the amount of urine excreted and its specific gravity. The coughing attacks occurred when the volume of urine was large and its specific gravity high, i.e.,when maximal amounts of sodium chloride were excreted. At the end of the experiment, when the diuresis had considerably abated, despite the continued increase in specific gravity of the urine there was no cough.

As Sel'kurt and Post [5] showed, when large amounts of 5% sodium chloride are injected into the blood stream, there is a rapid increase in the excretion of sodium with the urine. Sodium excretion increases as a result of a marked increase in filtration, which is caused by a reduction in the oncotic pressure of the plasma, an increased blood volume, and some increase in blood pressure. The increased sodium concentration and more rapid filtration causes a considerable increase in the amount of filtered sodium; consequently the reabsorption of of sodium is somewhat increased and the amount per 100 ml of filtrate may be actually somewhat reduced. All these factors facilitate the rapid excretion of sodium with the urine. Under a sodium load the kidneys do more work in concentrating the urine against a high osmotic gradient [2].

It is difficult to say which of the factors are concerned in the reflex responses. Our experiments appear to demonstrate only that when large amounts of sodium chloride are excreted with the urine, the interoceptors of a reinnervated transplanted kidney are stimulated, so that a typical "vagus" response, as shown by a coughing attack, is induced.

SUMMARY

In dogs, one kidney was transplanted into the neck and reinnervated by Shpuga's method; the central end of the sectioned vagosympathetic nerve was joined to the peripheral end of renal nerves, and the change in respiration due to different stimuli was studied. A reflex change occurred when the kidney was stimulated mechanically or by induced current, or when the renal pelvis was dilated by fluid under pressure or irrigated by sodium, potassium, calcium, or magnesium chloride solutions. By this method it was possible to experiment on the kidney interoceptors in chronic preparations.

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* Original Russian pagination. See C.B. translation.

CHANGES IN THE HUMAN ELECTROCARDIOGRAM ON BREATHING OXYGEN UNDER PRESSURE AND THEIR RELATIONSHIP TO A COMPENSATING PRESSURE APPLIED EXTERNALLY TO THE BODY (VECTOR ANALYSIS)

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Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50, No. 12, pp. 7-11, December, 1960
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Respiration under increased pressure was first studied in Russia by P. P. Einbrodt in 1860 [5]. Recently it has been widely used both in the practice of aviation, and clinically. It has been shown that breathing under pressure is well tolerated in man provided the excess pressure in the lungs does not exceed 25-30 mm Hg. Higher pulmonary pressures may be tolerated for a certain time but only when an equal external compensating pressure is applied. In practice various forms of compensating devices are used, the best of which is the height-compensating suit [4].

The purpose of the present work has been to study the effect of the value of the compensating pressure on the electrical activity of the myocardium, while the subject was breathing under increased pressure.

The problem has been studied by G. V. Altukhov and N. A. Agadzhanyan [1] by V. S. Gurfinkel', D. I. Ivanov, A. E. Ivanov, and V. B. Malkin [3]; a special study of this problem has also been made by Mahoney and Kennedy [6], who showed that an increase of pulmonary pressure from 30 to 60 mm Hg without external compensation causes sinus arrythmia, an increase in the Q-T interval, and a rotation to the right of the electrical axis of the heart (α) and of the vectors AQRS and AT. When the excess pressure in the lungs is 50-100 mm and a counter-pressure is applied from outside, there is practically no change in the ECG; at a pressure exceeding 100 mm Hg, a compensating suit is no longer effective in preventing the ECG changes already described, which occur both when the experiment is conducted either at ground level or in a rarefied atmosphere corresponding to a height of 20,000 m above sea level.

We have carried out two series of observations both at normal atmospheric pressure and at a pressure corresponding to a height of 20,000 m. To breathe under excess pressure, an oxygen device was used which produced an intrapulmonary pressure of 145 mm at a "height" of 20,000 m; it incorporated a hermetically sealed helmet and a special compensating suit type VKK-2 "M" which allowed pressure to be applied separately to the abdomen thorax, thighs, shoulders, lower legs, forearms.

The ECG was recorded by means of the 3 standard leads and 2 chest leads (CR_{II} and CR_{V}). A six-beam oscillograph was used, and simultaneous recording was made of the respiratory movements of the thorax, the electromyogram of the intercostal muscles, and of the oblique abdominal muscle. First a study was made of the effect of different values of externally applied pressure while the subject breathed under excess pressure; next the effect was tried of increasing the pressure on different parts of the body. In both experiments the excess pulmonary pressure was kept constant for each investigation. For different "ground" conditions it was made equal to 55, 104, 126, and 135 mm Hg, and at "high altitudes" it was kept at 104 mm mercury.

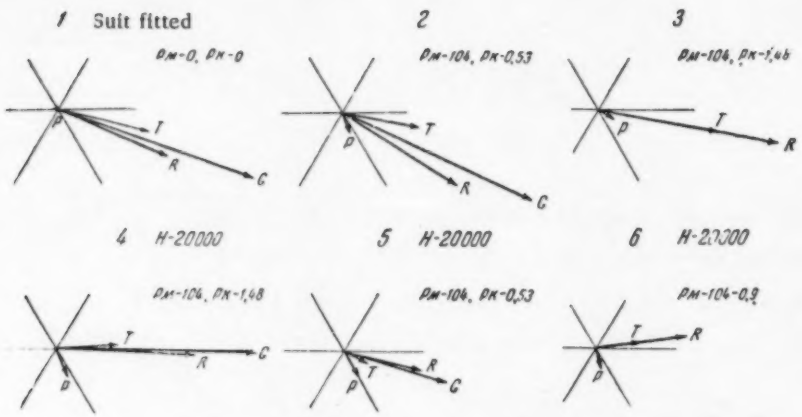

Fig. 1. Frontal vector cardiogram. Subject P-in, February 5, 1957. 1, 2, 3) "Ground level" conditions; 4, 5, 6) "high altitude" conditions (20,000 m): P_m) excess pulmonary pressure (in mm Hg); P_k) external body pressure: 0.53-70 mm less than the intrapulmonary pressure; 1.48) equal to intrapulmonary pressure; 0.9-50 mm Hg less than intrapulmonary pressure; P) the AP vector; R) AQRS (AR) vector; T) AT vector; g) "auricular gradient."

In the first set of experiments, the pressure within the pressure suit was varied so as to apply a pressure to the body of 70, 50, and 30 mm Hg more or less than that in the lungs. At the start, a pulmonary pressure of 60 mm was established without external compensation. Recordings were then taken for the same interpulmonary pressure after the pressure suit had been put on but no pressure established in it. Then a particular intrapulmonary pressure was established, and the pressure in the suit changed as described above. The same sequence was observed at both "ground level" and "high altitudes."

In the second set of experiments, pressure was applied separately to the abdomen, thorax, etc. At "ground level" and at an arbitrary pulmonary pressure of 55 mm Hg, a pressure equal to the internal value was applied alternately to the areas named above. Then, after the subjects had rested, a second series of observations were made at an intrapulmonary pressure of 104 mm Hg, and the opposing external pressure was reduced (usually to zero) over the same areas. "High altitude" studies were carried out, just as at "ground level", at an excess pulmonary pressure of 104 mm. A total of 69 studies were made, 49 in the first and 20 in the second series.

RESULTS

The results of the first set of the experiments showed that at excess pulmonary pressures of from 55-136 mm Hg and an equal externally applied pressure, at "ground level" the electrical cardiac axis (alpha), and the AP, AQRS, and AT vectors and the "auricular gradient" (G) showed some deviation to the right, without any special separation, so that a rotation of the heart to the right about a sagittal axis and a deviation of the apex backwards were indicated. We think that in this case positional changes in the ECG occur as a result of the more vertical position of the heart in the thorax, which is brought about by a fall in the diaphragm due to the increased intrapulmonary pressure.

Changes in lead III were caused by an excess pressure of 60 mm in the lungs without external compensation. Then the P_{2-3} waves were increased and changed in shape, the AP vector was rotated to the right through 15-40°, and somewhat increased. The AQRS, AT and G vectors were also deviated to the right away from their original position by 10-35°. The amplitude of the auricular vectors was usually reduced, AQRS by 10-15μ vs, AT by 5-10μ vs, a result which indicated a relative oxygen deficiency of the myocardium.

Under these conditions, the pressure suit caused considerable alterations in the ECG. Even putting on the suit without introducing any pressure reduced the deviation to the right of the cardiac vectors.

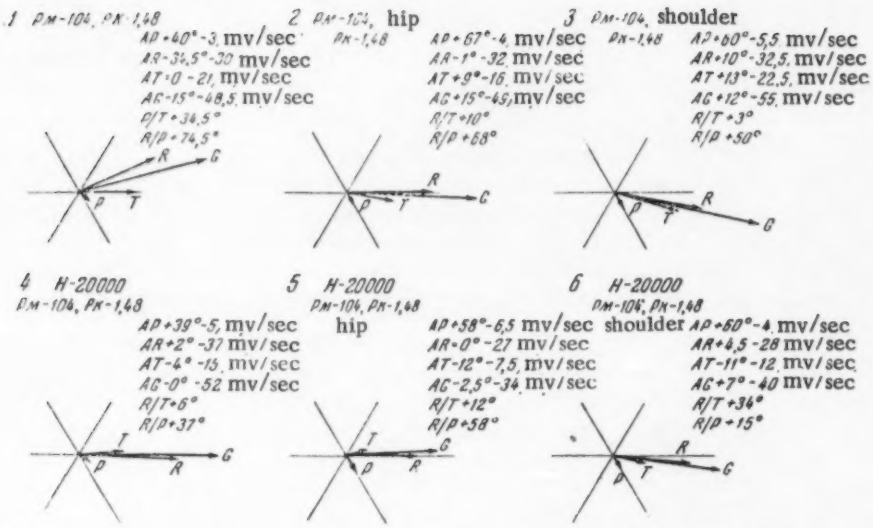

Fig. 2. Frontal vector cardiogram. Subject P-in, March 26, 1957. 1, 2, 3) "Ground level" conditions; 4, 5, 6) "high altitude" (20,000 m) conditions. Other indications as in Fig. 1.

At "ground level" increasing or decreasing the external pressure by 30-50 mm relative to the intrapulmonary pressure caused no significant alteration of the changes of the ECG induced by the increased pulmonary pressure, i.e., the typical changes already described as due to breathing air at an increased pressure remained. However, the R/T (AQRS/AT) and R/P (AQRS/AP)* ratios were greater the smaller the body pressure in relation to the intrapulmonary pressure (Fig. 1, 2). These ratios indicate the spacial relationships of the vectors representing excitation and recovery of the ventricular myocardium (R/T) as well as the spatial relationships of the vectors of the potentials of the ventricular and auricular myocardia (R/P).

In working at "ground level" we failed to observe any appreciable changes in the P–Q, Q–T intervals or in the systolic index. In certain cases there was an increase in the S–T_{2-3} line, which might be interpreted as an indication of the preponderance of vagal tone, and indications of incomplete block of the right fork of the bundle His, which can be explained as due to a relative overload of the right ventricle.

Changes in the ECG in the corresponding experiments made at a reduced atmospheric pressure (at an "altitude" 20,000 m), were basically the same, except that the reduction in the ventricular vectors was better shown. Then, the smaller the pressure in the suit, the smaller were the vectors, particularly the AT vector. There was also considerable separation in space of the AP and AQRS vectors (Fig. 1, 5, and 6).

Under "ground level" conditions, in the second set of observations when there was a 55 mm excess pulmonary pressure, application of an equal external pressure to the abdomen, whose muscles play an important part in breathing out against an increased pressure caused alterations in the ECG; there was some separation of the vectors in space and a rotation, particularly of the AP vector through 20-30° to the right. There was also an increase in the R/T and R/P ratios. Applying the same pressure to the thorax or limbs caused only the same kind of changes in the ECG. The amplitude of the vectors, the duration of the P–Q and Q–T vectors, and the systolic index scarcely differed from their initial values. The changes in the ECG described are due to the position of the heart which is altered by the raised intrapulmonary pressure.

At an excess pulmonary pressure of 104 mm or above, and when an equal counter pressure was applied externally, the reduction of the pressure of the different areas, either at "ground level" or at "high altitudes" induced more complex ECG changes (Fig. 2). The amplitude of the vectors, particularly of the AT vector was

* The ratio which we have proposed [2].

considerably reduced. The AP vector deviated to the right, there was a separation of the AP and AQRS vectors in space and an increase of the R/P ratio.

The fact that there was also a separation of the AQRS and AT vectors and a leftward rotation of the electrical axes showed that in addition to positional ECG changes there were also circulatory disturbances of the myocardium.

The most marked changes in the ECG occurred when the external pressure in the region of the abdomen was reduced, and the effect was even more marked for the thighs (see Fig. 2, 5). There was then not only a considerable separation in space of the auricular and ventricular vectors, but there was also a marked increase in the number of cardiac contractions, the $S - T_{2-3}$ interval fell below the baseline, and there was an increase of 14-28% in the systolic index. These changes show that when air is breathed under increased pressure, the counter-pressure applied to the thighs, belly, and thorax plays an important part in preventing circulatory disturbances.

Vector analysis of the ECG therefore allows us to conclude that when a subject breathes air at an increased pressure, there is an overload first of the right auricle, and then of the whole of the right heart. This method enables positional ECG changes to be distinguished from functional disturbances of a particular region of the heart. When the pulmonary pressure is increased to 136 mm, variations in the value of the compensating pressure up to 50 mm of mercury have no appreciable effect on the condition of the myocardium. If under these conditions insufficient compensation is applied to the abdomen or thighs, there is a marked disturbance of the cardiovascular system, and of the myocardium in particular. The ECG changes serve as an important objective indication of the extent to which a particular individual may tolerate an increased intrapulmonary pressure.

SUMMARY

Two series of observations were made in which the atmospheric pressure was reduced to a value corresponding to an altitude of 20,000 m. In the first, a study was made of the effects of a counter pressure applied externally to the body; in the second series the pressures were applied to separate areas of the body. When the excess pressure in the lung reached 136 mm Hg, and when an equal counter pressure was applied externally, the electrical axis and cardiac vectors deviated to the right. Variations of the external pressure up to 50 mm Hg applied while the intrapulmonary pressure is held constant, do not by themselves cause any significant changes in the ECG. When the pressure applied to the abdomen and thighs is insufficient, the cardiac vectors diminish, and circulatory disturbances occur. Vector analysis of the ECG shows that during respiration under increased pressure, the first changes occur in the right heart.

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THE LOCAL REFLEX COMPONENT IN THE CARDIAC RESPONSE TO RAISED INTRAPULMONARY PRESSURE AND TO INCREASED PRESSURE IN THE VENA CAVA

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Besides the central nervous system, the local viscero-visceral reflexes also play an important part in exerting control over a number of organs. Little work has been done until now to determine the importance of such reflexes in the control of cardiac activity.

In a heart-lung preparation, cardiac contractions increase as described by "Starling's Law" [5] when pressure in the vena cava is raised, and this effect results from the stretching of the cardiac muscle fibers due to the increased venous return into the right auricle during diastole. The marked reduction in cardiac output caused by increased intrapulmonary pressure is due to the increased resistance of the vessels of the lesser circulation.

However, there have been reports that the above explanation is incomplete. V. P. Demikhov [1] has pointed out that the small increase in pressure in the vena cava which is observed to induce an increase in cardiac contractions could not cause an appreciable mechanical extension of the thick muscle walls of the ventricles. G. P. Konradi [3] has quoted the experiments performed in his laboratory by A. A. Morozov, in which Ringer's solution containing novocaine passed through the heart eliminated the increase in systolic volume following increased venous pressure. The response to the injection of adrenaline was still maintained, indicating that the myocardium itself was not paralyzed by the novocaine. It was therefore proposed that the increased cardiac contractions following increased pressure in the vena cava must be brought about through nervous structures in the heart.

Essentially, on such a view, local reflexes would be involved in bringing about the response to increased pressure in the veins and distension of the auricular fibers, as described in Starling's Law. As far as the depression in the cardiac activity through increased pulmonary pressure is concerned, the reaction may be modified by a number of drugs; it would appear therefore that the phenomenon cannot be explained in terms of the increased resistance of the lesser circulation alone.

The object of the present investigation was to determine the part played in these reactions by central reflexes.

V. V. Zakusov and his co-workers [2] have shown that besides blocking transmission through sympathetic and parasympathetic ganglia, ganglion-blocking agents also depress viscero-visceral reflexes to a considerable extent. To depress the local reflex component to increased venous and pulmonary pressures we therefore used 0.1-2 mg per kg body weight of the ganglion-blocking agent arphonade.

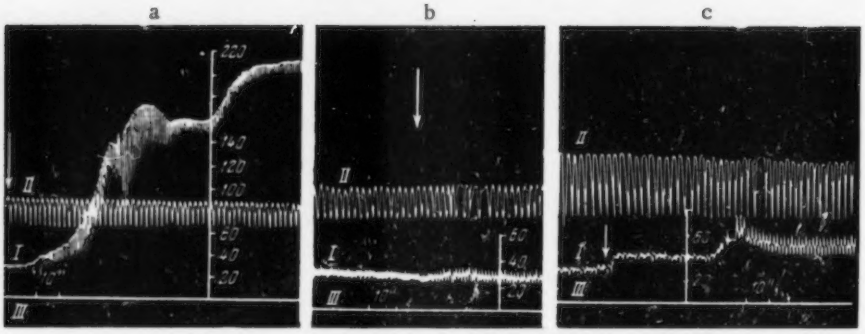

Fig. 1. Arterial pressure changes in a heart-lung preparation in response to the injection of 2 ml per kg of blood through a catheter into the right auricle (moment of injection shown by arrow). a) Initial response; b) after giving 1 mg per kg arphonade; c) after giving 5 mg per kg cysteine; I) blood pressure; II) intrabronchial pressure; III) zero blood pressure line. Experiment No. 28, August 23, 1959; weight of dog 10.5 kg.

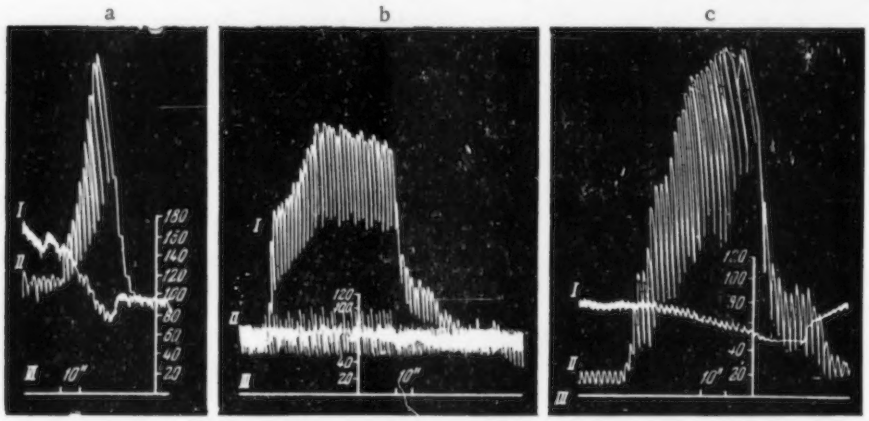

Fig. 2. Arterial pressure changes in a heart-lung preparation in response to increased intrapulmonary pressure. a) Initial response; b) after injecting 1 mg per kg arphonade; c) after injecting 5 mg per kg cysteine. Curves as in Fig. 1. Experiment No. 14, March 21, 1959; weight of dog 6.5 kg.

METHOD

A heart-lung preparation was made under morphine-thiopental anesthesia using the modification described by Yu. M. Gal'perin. A cannula was introduced into the subclavian artery, catheters were inserted into the azygous vein, the innominate artery and into the aorta (below the origin of the subclavian artery), and ligatures were applied. The cannula in the subclavian artery was connected to a pressure vessel at a height of 120 cm above the operating table. After withdrawing blood equal to 1.5-2% of the body weight, the ligatures were tightened, and owing to the cardiac contractions blood continued to flow from the left ventricle into the pressure vessel. After 5-8 min., when impulses from the central nervous system to the heart had been cut off, spontaneous contractions were produced which could be recognized from the nature of the blood pressure curve recorded by a mercury manometer, connected to the subclavian artery. When the cardiac contractions had become fairly constant, the pressure vessel was disconnected, and the following operations carried out: 1) a standard amount of blood (2 ml per kg body weight) was introduced into the right auricle through the catheter previously connected to the azygous vein; 2) the intrabronchial pressure was rapidly raised to 250-300 mm of water. The responses were recorded, and then the same operations were repeated after injecting 0.1-2 mg per kg of arphonade. When, during the experiment, the arterial pressure fell the pressure was connected to the preparation for 30 sec.; as a rule there was then

a considerable increase in the strength of the cardiac contractions and in arterial pressure. We performed a total of 12 experiments on heart-lung preparations of dogs weighing 6.5 - 22 kg.

RESULTS

The initial responses to the introduction of 2 ml per kg of blood into the right auricle and to increasing the pulmonary pressure to 200-300 mm Hg were all of the same type, and varied only in strength (Figs. 1a and 2a).

The introduction of up to 10 mg per kg body weight of arphonade into the coronary circulation caused no impairment of cardiac activity. The contractions were slightly increased and the frequency remained unchanged.

After injecting arphonade, the heart consistently changes its response to the measures applied. The reaction to the infusion of 2 mg per kg weight of blood into the right auricle was greatly reduced and delayed. The blood pressure showed a very slight rise, or else was unchanged, and in four of the 12 experiments it was actually reduced. As a rule there was a greater range of arterial pressure variation, which, however, did not occur for 8-15 sec. after the blood had been given (Fig. 1b), whereas before arphonade was injected the effect occurred within 1-2 sec.

An increase of intrapulmonary pressure after the infusion of arphonade caused some change in the nature of the recorded cardiac contractions; however, the blood pressure remained at the previous level, or was even somewhat increased (Fig. 2b).

Kh. S. Koshtoyants and his co-workers [4] showed that sulfhydryl groups play an active part in nervous excitation in general and in the transmission of nervous excitation in ganglia in particular; in six experiments therefore, after giving arphonade, we injected 5 - 10 mg per kg of cysteine in order that this sulfhydryl-group donor should come in contact with local reflex arc. After giving cysteine, the reaction to raised intrapulmonary pressure was approximately the same as it was initially (Fig. 2c); only in certain cases a greater and more prolonged increase in intrapulmonary pressure than had been required previously was needed to cause a drop in arterial pressure. The response to infusing blood after giving cysteine never reached the original intensity; however, it was greater than the response which occurred after arphonade had been given (Fig. 1c). Then the change in cardiac activity in response to an increase in the pressure in the vena cava occurred immediately after introducing the blood into the right auricle.

The cardiac response induced by arphonade is probably not due to any direct action on the coronary vessels, because no fall in coronary arterial pressure occurred after it had been injected. Further, the fact that its effects could be reduced or eliminated by means of cysteine also shows that arphonade affects the nervous structures of the ganglion directly.

The results of the experiments indicate that drugs which cause a change of conductivity in local intracardiac and cardiopulmonary reflexes have a definite influence on the responses induced by increase of intrapulmonary pressure and pressure in the vena cava. It would therefore appear that local reflex action plays an important part in the response described by Starling's Law, and in the response to increased pulmonary pressure.

SUMMARY

Experiments were performed on canine heart-lung preparations. Injections were given into the coronary circulation of 0.1-2 mg/kg body weight of arphonade, a ganglion-blocking agent which depresses local viscero-visceral reflexes; it was found that cardiac reactions to increased blood pressure in the vena cava (Starling's Law) and to a rise of intrapulmonary pressure were depressed. Injecting 5 - 10 mg/kg body weight of cysteine, which is a sulfhydryl-group donor partially restored the initial reactions. The results obtained indicate that local reflexes play an important part in the reactions described.

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THE EFFECT OF THYROXIN ON GASEOUS EXCHANGE IN WHITE RATS OF VARIOUS AGE GROUPS

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The injection of thyroxin is known to increase the gaseous exchange in normal animals, and the effect occurs after a latent period of 18-24 hr. There are certain indications that the effect obtained depends on age. V. N. Nikitin, R. I. Golubitskaya, L. A. Dryuchina and others [6] have shown that in old rats the response to thyroid hormone is reduced. T. O. Dzgoeva [1] found that as little as 14 μg/100 g has no effect on the gaseous exchange of two-week or one-month rats, whereas the same doses increase it considerably in animals one year old. Old rats react more weakly than the one-year-olds to the same dose of thyroxin.

It has also been shown [2, 7] that there are marked seasonal variations in the rate of gaseous exchange in rodents, even when temperature and other conditions are maintained constant.

In the present work we have attempted to determine the effect of various doses of thyroxin on gaseous exchange of animals of various age groups, while taking into account seasonal variations in the basic metabolic rate.

METHOD

Rats of three age groups were used: Adults of 1–2 years weighing 180–240 g, sexually immature animals 22–35 days old weighing 35–49 g, and old rats of 2–3 years weighing 290–350 g.

Gaseous exchange was determined by the closed chamber method using M. N. Shaternikov's method for small animals as modified by N. A. Isichenko [3]. The experiment was started 16–18 hours after the rats had last fed. Gaseous exchange was measured before, and 18–20 hr after 50 and 200 μg/100 g had been injected subcutaneously. The absorption of O_2 and the liberation of CO_2 per hour were referred to 100 g body weight. Gaseous-exchange measurements in the animals of different age groups were continued for one year, and 70 animals were used.

RESULTS

Seasonal variations of gaseous exchange were particularly marked in the sexually immature rats. Absorption of oxygen in winter was almost twice as great as in summer (the increase being 0.2 liters/hr). The increased oxygen absorption in winter was less marked in the old and middle age groups, the winter value exceeding the summer by 0.03 and 0.04 liters/hr respectively.

The injection of 50 μg/100 g of thyroxin into rats of the middle-age group in summer caused no appreciable change in gaseous exchange. In autumn the same dose increased oxygen absorption by 0.03 liters/hr, and the liberation of CO_2 by 0.02 liters/hr, and there was an insignificant reduction of the respiratory quotient (Table 1). When 200 μg/100 g of thyroxin was given in either summer or winter, oxygen absorption was increased by 0.05 liters per hr, and CO_2 liberation by 0.03 liters/hr.

TABLE 1.

Changes in Gaseous Exchange in Rats of Middle Age Before (I) and After (II) the Injection of 50 and 200 μg/100 g of Thyroxin (average values)

No. of animals	Wt. (in g)	Month	Dose (in μg/100 g)	O_2 (100 liters per hr)		CO_2 (100 liters per hr)		Respiratory quotient	
				I	II	I	II	I	II
7	220	July	50	0,16	0,175	0,14	0,16	0,87	0,91
7	208	October	50	0,17	0,20	0,15	0,17	0,88	0,85
7	235	July	200	0,15	0,19	0,14	0,17	0,93	0,89
8	218	Dec.	200	0,19	0,24	0,18	0,21	0,95	0,87

TABLE 2.

Change in the Gaseous Exchange in Sexually Immature Rats Before (I) and After (II) the Injection of 50 and 200 μg/100 g of Thyroxin (average values)

No. of animals	Wt. (in g)	Month	Dose (in μg/100 g)	O_2 (100 liters per hr)		CO_2 (100 liters per hr)		Respiratory quotient	
				I	II	I	II	I	II
5	48	July	50	0,20	0,19	0,20	0,18	1,0	0,95
5	41	"	200	0,22	0,22	0,20	0,20	0,92	0,92
10	37	Sept.	200	0,31	0,31	0,29	0,27	0,93	0,87
12	39	Dec.	200	0,42	0,48	0,39	0,42	0,93	0,89
3	35	Oct.	200	0,35	0,40	0,32	0,35	0,91	0,87
3	35	"	200	0,41	0,38	0,36	0,35	0,88	0,92

TABLE 3.

Change in Gaseous Exchange in Old Rats Before (I) and After (II) Injecting 50 and 200 μg/100 g of Thyroxin (average values)

No. of animals	Wt. (in g)	Month	Dose (in μg/100 g)	O_2 (100 liters per hr)		CO_2 (100 liters per hr)		Respiratory quotient	
				I	II	I	II	I	II
7	305	August	50	0.16	0.16	0.15	0.15	0.94	0.94
6	283	October	50	0.16	0.18	0.14	0.15	0.88	0.83
6	290	July	200	0.14	0.16	0.13	0.14	0.93	0.88
6	319	Dec.	200	0.17	0.20	0.16	0.18	0.94	0.90

Thus in rats of medium age, small doses of thyroxin cause seasonal changes in reactivity: In summer they react weakly, but in autumn gaseous exchange is increased. When large doses are given, however, no seasonal changes in the extent of the reaction occur.

Unlike the rats of medium age, the sexually immature animals showed no change in the absorption of O_2 or the liberation of CO_2 when 50 or 200 μg/100 g were given in summer or autumn. If 200 μg/100 g was given to the sexually immature animals in winter, oxygen absorption was increased and the respiratory quotient depressed.

TABLE 4.

Change in Gaseous Exchange in Rats Before (I) and After (II) the Injection of 200 μg/100 g of Thyroxin as Related to the Original Basal Metabolic Rate

No. of animals	Wt. (in g)	Month	Dose (in μg/100 g)	O_2 (100 liters per hr)		CO_2 (100 liters per hr)		Respiratory quotient	
				I	II	I	II	I	II
1	245	July	200	0.13	0.22	0.12	0.19	0.22	0.86
2	210	"	200	0.19	0.21	0.17	0.18	0.89	0.86
3	328	October	200	0.13	0.20	0.13	0.15	1.0	0.75
4	360	"	200	0.22	0.21	0.17	0.16	0.77	0.76

It should be noted that the change in gaseous exchange in sexually immature rats produced when the thyroxin injection was given in October, between seasons, was variable, and the gaseous exchange rate might be either increased or decreased. The change was related to the initial basal metabolic rate, the gaseous exchange rate being increased when it is initially low, and vice versa (Table 2).

Like the sexually immature rats, the old animals did not react to 50 μg/100 g of Thyroxin given in summer, but showed some increase in metabolic rate when the same amount was given in autumn. A dose of 200 μg/100 g caused some increase in gaseous exchange rate, but it is not so marked as in rats of the medium-age group (Table 3).

To sum up, we may note first of all that seasonal variations in metabolic rate were more marked in the sexually immature than in the adult or old rats. The change in gaseous exchange caused by the injection of thyroxin depended also on the season; it was weaker in summer and more marked in winter, the difference being particularly well shown in the younger animals. The greater increase in gaseous exchange in these animals in response to thyroxin given in winter is probably adaptive, and associated with the necessary chemical heat regulation.

The sexually immature animals reacted less to thyroxin than did those of the other eight groups: No effect was produced by a dose of 50 μg/100 g, and in summer there was no response to one of 200 μg/100 g, whereas the same dose at the same season caused an increased gaseous exchange in the adults. The older animals reacted less to thyroxin, but more than did the sexually immature rats.

It is important to note that at high metabolic rates which were not due to seasonal influences there was a definite relationship between the initial rate and the response to thyroxin. When the initial rate was high, the response to thyroxin was less marked (Table 4); in many animals there might be even a paradoxical effect, when the absorption of oxygen was reduced. This phenomenon has been reported previously [4, 5].

SUMMARY

Seasonal variations of the BMR were established in rats; they were more pronounced in sexually immature than in adult or old animals. The sexually immature rats reacted less to thyroxin than did the adult or old animals. The reaction to thyroxin was greater in winter. In a number of experiments the response was diminished or even paradoxical when the initial metabolic rate was high.

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A VESTIBULO-PALPEBRAL REFLEX

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According to published reports, impulses from the vestibular apparatus exert a marked influence on the development of the human nervous system during both the pre- and post-natal periods. The vestibular apparatus is completely formed in a fetus of 6-7 months, and at birth it is associated with various effectors, so that when it is stimulated contractions occur in the muscles of the limbs, neck, trunk, and eyes, and there are changes in blood pressure and in the blood supply to the brain [3, 4]. It would therefore be expected that there might be some connection between the vestibular apparatus and the muscles which open the eyelids.

It is known that during the first few days after birth it is very difficult to induce the eyes to open during sleep, particularly in premature infants. Clapping, light shaking, touching the lips or cheeks, and shining light on to the eyes very seldom causes the lids to open. However, it appears that the opening may be readily elicited by appropriate stimulation of the vestibular apparatus.

METHOD

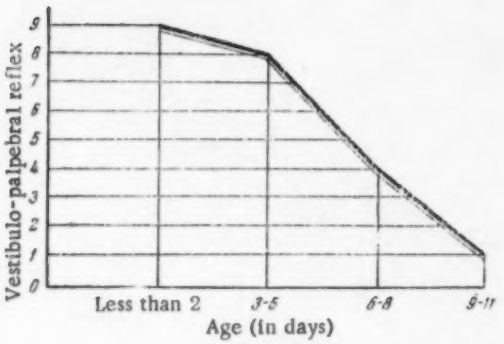
We studied 87 infants born at term, and 8 which were premature. The former were divided into 4 groups aged up to 2 days, from 3-5, 6-8, and from 9-11 days. To stimulate the semicircular canals the child was picked up as it lay on its back and was then rapidly rotated so as to face downwards. When such an influence was applied to the vestibular apparatus, the muscles opening the eyelids contracted.

RESULTS

In the group aged 2 days or less the reaction occurred in 9 out of the 10 infants, in the group aged from 3-5 days in 8, in the 6-8 day group in 4, and in the group aged 9-11 days, only one child responded. The opening of the lids was not accompanied by any generalized motor reaction, as it is on awaking, but the response was purely local and lasted from 1-5 sec. The eyelid movement differed in the different children; in most the opening was clearly seen, and in some, although the muscles raising the lids contracted, the edges of the lids remained in contact. Cases where rotation of children only a few hours old failed to cause any opening were usually those where the lids were greatly swollen, and evidently could be opened only with great difficulty.

We thus found a reflex which caused the lids to open when the vestibular apparatus was sufficiently stimulated, and we have called it the vestibulo-palpebral reflex.

As we have shown, this reflex may be elicited during the first hours of life, but subsequently, like other phylogenetically ancient reflexes, it disappears, though earlier than, for example, the Palmar reflex, or the Babinski or Moro embrace, or the Rossolimo, etc. reflexes. In premature infants, by the 6th-8th day of life the reflexes are less stable, and by the 9th-12th day they are absent entirely. The diagram shows the gradual disappearance of the reflex in children of the different age groups.



The disappearance of the reflex.

In premature infants, the reflex is maintained for considerably longer, and we have even observed it during the second month of life. In the premature group the reflex occurs, as it does in infants born at term. The difference is that in premature infants, during the first few days after birth, contractions which cause only a very small opening of the lids are more frequently observed. If however, the vestibular apparatus is stimulated 3-4 times in succession in the manner described, quite a wide opening of the lids is caused.

As can be seen from the diagram, in children born at term the reflex fails by the 9th-11th day. It is true that the eyelids may be induced to open by rotating older infants but the reflex is then not local, the child wakes up, and the reflex often occurs immediately after the child is picked up, so that it may be due to tactile stimuli. In children less than 11 days old, and in premature infants, the reflex is truely local.

Therefore, appearance of a temporary connection at an early stage, in the development of the individual, between the vestibular apparatus and the muscles of the eyelid, represents yet one more example of a simple reflex which disappears quite early in life. The relationship must be looked on as a vestigial association whose transient manifestation indicates that evolution proceeds not by the complete elimination of such connections but by superimposing upon them new functional relationships [5] which are in keeping with the new conditions of existence.

An ontogenetic mechanism which deserves particularly careful study is that which causes the disappearance of such inherited established connections. The disappearance of such reflexes as the Palmar, Babinski, Moro and other reflexes is normally attributed to the restraining effect of the cerebral cortex. M. I. Astvatsaturov [1] has shown that reflexes which are caused to disappear at an early age through the action of the cerebral cortex pass into a latent state. This view is confirmed by the appearance under pathological conditions in adults of the Babinski, Rossolimo, Mendel-Bechteraw, Robinson, and other reflexes.

Results obtained on decorticate animals, and observations on anencephalic and feeble-minded children are evidence of the action of the cerebral cortex in causing the elimination of various simple reflexes. The tonic neck and labyrinthine reflexes are found in their most complete form in decerebrate animals, and are particularly clearly shown in anencephalic children.

A detailed study of animal development, A. A. Volokhov [2] has shown that a number of specialized reflexes disappear during the post-natal period, and that effective elimination of the cortex, either by extirpation or by reducing the partial pressure of oxygen, releases the "previously inhibited specialized reflexes."

These facts further substantiate the view of the part played by the cerebral cortex in suppressing certain reflexes at an early stage. It is not however yet possible to explain the different times at which the separate reflexes are suppressed; some of them are inhibited early, some much later, and the total range covered is of the order of several months. In children born at term, the vestibulo-palpebral reflex described above disappears at the end of the second week, the Palmar reflex at the end of the fifth month, while the Babinski reflex can be elicited for the whole of the first year.

A further study of the vestibulo-palpebral reflex might establish a basis for its use in diagnosing vestibular disturbances at an early age, for determining how premature a birth has been, and possibly for determining individual features of the cortical activity which is manifested at such an early stage by the suppression of the reflex, as described.

SUMMARY

A study of 87 full-term and 8 premature infants revealed a connection between the vestibular apparatus and the muscles of the eyelids which is here described for the first time. The connection was shown by the opening of the eyes during the stimulation of the semicircular canals. The stimulation was effected by carefully raising

the infant and then suddenly turning it face downwards. The infant did not wake up, and the reflex, which we have called the vestibulo-palpebral reflex was strictly local, and not accompanied by any general motor reaction. It appeared during the very first hours after birth, but later it disappeared, as do other phylogenetically ancient reflexes, and earlier than, for instance, the Palmar or Babinski reflexes.

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CHANGE IN FUNCTIONAL STATE OF NEUROMUSCULAR PERIPHERY ON FORMATION OF SPINAL CORD DOMINANT

PART II. EFFECT OF SPINAL CORD DOMINANT ON MOTOR NERVE ACCOMMODATION

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It was shown in a previous communication [21] that when a dominant focus was formed in the flexor center of the spinal cord the excitation parameters (rheobase, chronaxie, threshold quantity of electricity and threshold energy) of the motor nerve and the muscle of the corresponding limb innervated by it were reduced and their values for the opposite limb were simultaneously increased.' In the present work we investigated the effect of a spinal cord dominant on the accommodation of the motor nerve. As far as we know, no investigations along this line have been carried out, though the accommodation parameter is a sensitive index of the functional state of tissue [8, 9, 12, 19] and reflects the state of its metabolism and surface polarization [6, 7, 20].

METHOD

We removed the cerebral hemispheres from the frog Rana ridibunda and prepared the semitendinous muscles, the peroneal and ulnar nerves (the peripheral ends of which were transected), and the motor branch of the sciatic nerve (its hind inferior branch) innervating the semitendinous muscle.* A graphic record was made of the contractions of the two semitendinous muscles. The dominant was created by subjecting the sensory peroneal nerve of the frog to the action of rhythmic subthreshold stimulations by an induction current of frequency 50 cps and strength 2 cm below the threshold). In the intervals between the action of the subthreshold stimulations we recorded the reflex contractions of the semitendinous muscles, and against this background we applied test stimulations of the ulnar of opposite peroneal nerves. The dominant was manifested in the development of a summation effect and in the distortion of the reciprocal relations between the centers of the antagonistic muscles. This was expressed in the enhancement of the contractions of the semitendinous muscles on test stimulations of the nerves.

The nerve accommodation was studied by means of exponentially increasing currents by D. I. Solandt's method [15]. The accommodation curves were plotted from the results of measurements of the stimulation thresholds with rise constants RC equal to 5, 10, 25, and 50 msec. To avoid excessive traumatization of the preparation (which affects the nature of its reflex activity) we did not employ exponential currents with a rise constant of more than 50 msec. The obtained stimulation threshold values, referred to the value of the rheobase v_0, were plotted on the y-axis and the rise constants of the stimulating current were plotted on the x-axis. The rate of

*The method was fully described in a previous paper [21].

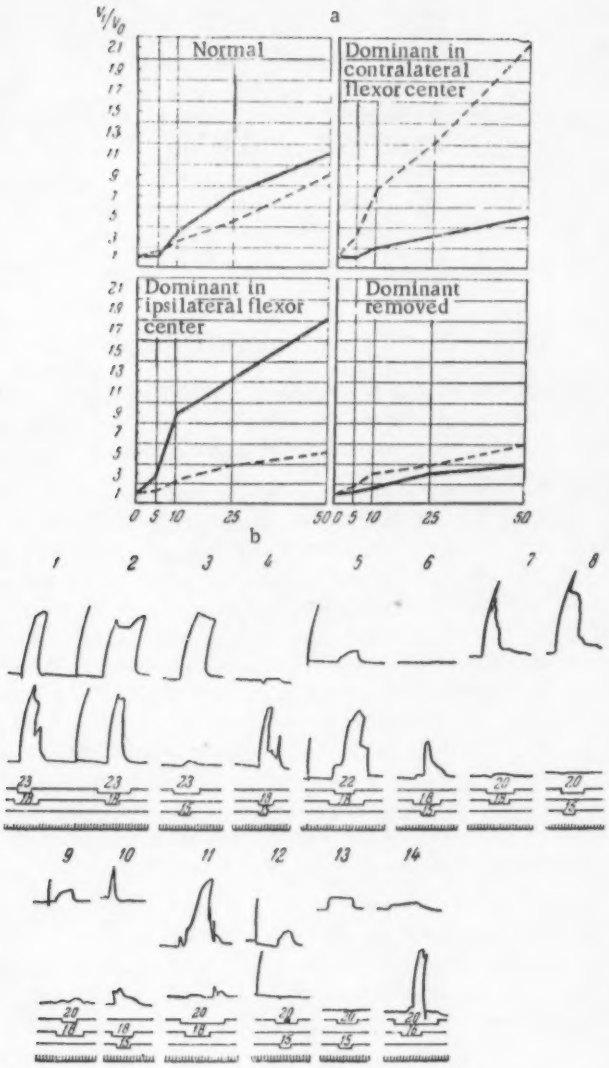

Fig. 1. Change in accomodation curves of motor nerve on formation of dominant in flexor centers of spinal cord (Expt No.78, August 8, 1957). a) Accommodation curve of ipsilateral motor nerve (hind inferior branch of sciatic nerve; solid line). On the y-axis) v/v_0; on the x-axis) rise constants (in msec); b) kymogram of same experiment: frames 1, 2,3, 4) reflex contractions of semitendinous muscle at start of experiment, before formation of dominant; frames 5, 6, 7, 8) the same on formation of dominant in contralateral flexor center by subthreshold stimulations of contralateral peroneal nerve (frequency 50 cps, strength 2 cm below stimulation threshold); frames 9, 10) the same after disappearance of dominant; frames 1, 2, 3) the same after formation of dominant in ipsilateral flexor center by stimulation of ipsilateral peroneal nerve by subthreshold current; frame 14) the same after disappearance of dominant. Meaning of curves (from top to bottom): myogram of reflex contractions of ipsilateral semitendinous muscle; the same for contralateral semitendinous muscle; stimulation marks for ipsilateral peroneal nerve; the same for contralateral peroneal nerve; the same for ulnar nerve; time marks (1 sec). Numbers above stimulation marks—distance between coils (in cm).

accommodation, corresponding to the initial slope of the accommodation curve, was calculated from A. V. Gill's formula [5]: $1/\lambda = \frac{v - v_0}{v_0 \cdot a}$ for the rise constants of 5, 10, and 25 msec.

A total of 97 experiments were carried out—13 on an isolated nerve-muscle preparation and 84 on the intact animal. In some experiments we determined the rheobase and chronaxie simultaneously with the measurements of the motor nerve accommodation and with the same electrodes. For the measurements we used a combined chronaximeter and adaptometer. We employed chlorided silver electrodes; the distance between them was 0.8-10 mm. The current direction was descending. The accommodation of the motor nerves of the two limbs was measured before formation of the dominant, during formation of the dominant, and after its disappearance.

RESULTS

The investigations showed that when a dominant was formed in the flexor centers of the spinal cord there was a marked increase in the rate of accommodation of the motor nerve of the limb in the centers of which the dominant had been elaborated. The rate of accommodation of the motor nerve of the opposite limb was reduced.

From a comparison of the accommodation curves for the motor nerve before and during formation of the dominant (Fig. 1a) we see that the slope of the accommodation curves for the motor nerve of the corresponding side increases both in the region of low rise constants (5 and 10 msec) and particularly in the region of larger values (25 and 50 msec). At the same time the accommodation curves for the motor nerve of the opposite limb have a more gentle slope than the initial curves before formation of the dominant. The kymogram of this experiment (Fig. 1b) shows that at first, before the application of the subthreshold stimulations, a reciprocal relationship existed between the antagonistic muscles—weakening of the contractions of the ipsilateral muscle on stimulation of the contralateral nerve and conversely (see Fig. 1b, 1, 2, 3, 4). The accommodation curves of the two motor nerves for this period of the experiment are shown in Fig. 1a (top left). After a ten-minute subthreshold stimulation of the contralateral peroneal nerve (in the interval between 4 and 5) a flexor dominant was elaborated in the centers of this limb. As the kymogram shows (see Fig. 1b, 5, 6), stimulation of the ipsilateral peroneal and ulnar nerves caused enhanced contraction of the contralateral muscle instead of inhibition. During the formation of the dominant the accommodation curve for the contralateral nerve became much steeper while that of the ipsilateral, on the other hand, decreased in slope, which led to a characteristic "scissors-like" position of the curves (see Fig. 1a, top right).

Later, on stimulation of the ipsilateral peroneal nerve in the same experiment a dominant was elaborated in the flexor centers of the ipsilateral limb (see Fig. 1b, 11, 12), and the relationship between the accommodation curves for the ipsi- and contralateral nerves was reversed (see Fig. 1a, bottom left). After disappearance of the dominant the two curves again became parallel and their angle with the x-axis was reduced (see Fig. 1a, bottom right).

In the majority of experiments we did not determine the whole accommodation curve but the rate of accommodation for two or three rise constants (5, 10, and 25 msec). Figure 2 shows the results of one of these experiments. After the fourth subthreshold stimulation of the ipsilateral peroneal nerve a dominant was formed in the flexor centers of the corresponding limb, (Fig. 2b, 5, 6) and, as the graph for this experiment shows (see Fig. 2a), the rate of accommodation of the ipsilateral motor nerve was greatly increased, especially for a rise constant of 5 msec (ten times; see Fig. 2a, 1). The rate of accommodation of the motor nerve of the opposite limb at this time for a 5 msec rise constant was halved (see Fig. 2a, 3). In the case of formation of a dominant in the contralateral flexor center the rate of accommodation of the contralateral motor nerve was greatly increased (13 times) as Fig. 3b, 3 shows. The rate of accommodation of the motor nerve of the opposite limb was halved (see Fig. 3b, 1).

In some experiments even repeated subthreshold stimulation of the sensory nerves (sometimes up to 15 times) did not lead to the formation of a spinal cord dominant; in this case we observed no appreciable changes in the rate of accommodation of the motor nerve. On the other hand, when the dominant was induced by previous chilling of the animal (without subthreshold tetanization), the rate of accommodation of the motor nerve was increased, just as in the case of the dominant elaborated by subthreshold stimulation of the sensory nerve. In both cases the increase in the rate of accommodation was greater, the stronger the manifestation of the dominant. In ten experiments with the dominant present we simultaneously measured the excitation parameters and rates of

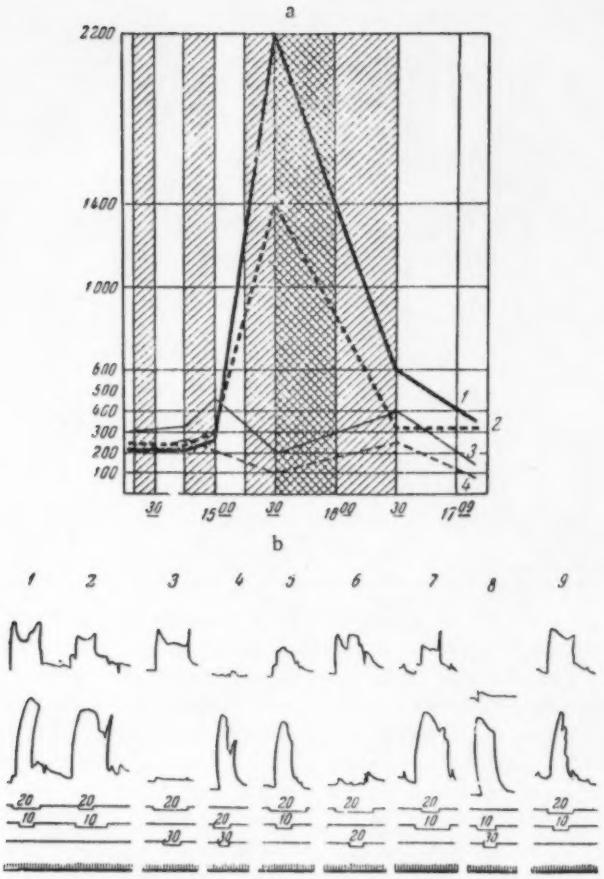

Fig. 2. Change in rate of accommodation of motor nerve on formation of dominant in ipsilateral flexor center (Expt No.32, December 26, 1958). a) Increase in rate of accommodation of ipsilateral motor nerve of semitendinous muscle. The ipsilateral peroneal nerve was again subjected for 10 min to subthreshold tetanization (hatched column) which resulted in formation of dominant (cross-hatched column); 1) rate of accommodation of ipsilateral motor nerve for rise constant 5 msec; 2) the same for rise constant 10 msec; 3) rate of accommodation of contralateral motor nerve for rise constant 5 msec; 4) the same for rise constant 10 msec; b) kymogram of same experiment; frames 1, 2, 3, 4) reflex contractions of semitendinous muscle before formation of dominant; frames 5, 6, 7) the same after formation of dominant in ipsilateral flexor center by stimulation of ipsilateral peroneal nerve (frequency 50 cps, strength 2 cm below stimulation threshold); frames 8, 9) the same after disappearance of dominant. Meaning of curves as in Fig. 1b.

accommodation of the motor nerves. Figure 3 shows the results of such an experiment. When the dominant arose in the contralateral flexor center (see Fig. 3c, 5, 6, 7, 8) the rate of accommodation of the contralateral motor nerve increased by a factor of 13 (see Fig. 3b, 3), the rheobase was reduced by a factor 2.5 and the chronaxie was

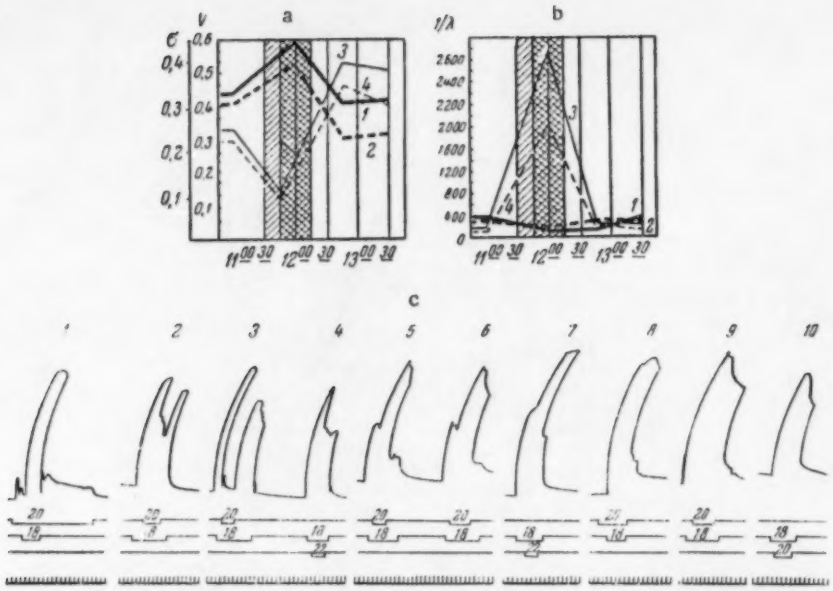

Fig.3. Change in rate of accommodation and excitability of motor nerves on formation of spinal cord dominant in contralateral flexor center (Expt No. 72, June 20, 1958). a) Change in excitability of motor nerve in presence of dominant; 1) rheobase of ipsilateral motor nerve; 2) chronaxie of ipsilateral motor nerve; 3) rheobase of contralateral motor nerve; 4) chronaxie of contralateral motor nerve; b) change in rate of accommodation of motor nerve in presence of dominant. Meaning of curves as in Fig. 2a; c) myogram of same experiment; frames 1, 2, 3, 4) reflex contractions of semitendinous muscle before formation of dominant; frames 5, 6, 7, 8) the same after formation of dominant in contralateral flexor center due to subthreshold tetanization of contralateral peroneal nerve (same conditions of experiment); frames 9, 10) reflex contractions after disappearance of dominant. Meaning of curves as in Fig. 1b.

halved (Fig. 3a). We observed similar relations in other experiments. As a rule the rate of accommodation was a more sensitive index of the change in the functional state of the nerve on formation of the dominant than the rheobase and chronaxie. Changes in the rate of accommodation usually preceded changes in nerve excitability, were more pronounced and were observed even in cases where the rheobase and chronaxie had still not changed significantly.

The table gives the results relating to the change in accommodation of the motor centers on formation of a dominant in the flexor center of one of the limbs. In 48 cases out of 54 the rate of accommodation of the corresponding motor nerve increased 200 to 400% on the average (in some experiments by a factor of ten or more) when the dominant appeared. In three cases out of the 54 we found no changes and in three cases we noted an insignificant reduction in the rate of accommodation. In 29 cases out of 42 the rate of accommodation of the motor nerve of the opposite limb was reduced, in nine cases it was unaltered, and in four cases it increased slightly.

When the dominant disappeared the rate of accommodation of the motor nerve was usually restored to its initial value. It should be noted that at the moment the dominant disappeared the rate of accommodation of the ipsilateral nerve was still below the initial level and only gradually attained it.

The changes observed in our experiments on the rate of accommodation of the motor nerve on induction of a spinal cord dominant were very similar in their nature and rapid reversibility to those usually observed in the development of electrotonus in the nerve [6, 7]. As we know, in the region of catelectrotonus the rate of accommodation increases markedly when the direct current is made and, when the current is broken, the rate of

Nature of Change in Rate of Accommodation of Motor Nerve on Formation of Dominant

On side of dominant				On opposite side			
total no. of observations	no. of observations with increased rate of accommodation: total	no. with indicated increase	increase in rate of accommodation	total no. of observations	no. of observations with reduced rate of accommodation: total	no. with indicated reduction	reduction in rate of accommodation
54	48	2	20:1	42	29	1	1:60
		4	15:1			1	1:15
		4	10:1			1	1:7
		4	8:1			1	1:4
		3	6:1			3	1:3
		3	5:1			7	1:2
		6	4:1			7	1:1,5
		6	3:1			8	1:1–1,5
		5	2:1				
		6	1,5—1				
		5	1—1,5:1				
	no. of observations with reduced rate of accommodation: total	no. with indicated reduction	reduction in rate of accommodation		no. of observations with increased rate of accommodation: total	no. with indicated increase	increase in rate of accommodation
	3	1	1:3		4	1	3:1
		2	1:1,5			1	2:1
						2	1,5:1
	total no. of observations with no change in rate of accommodation						
	3	—	—		9	—	—

accommodation at first falls below its initial value and is then restored to its initial level [6]. Similarly in our experiments, as we indicated above, the appearance of the dominant was accompanied by an increase in the rate of accommodation of the corresponding motor nerve, and the disappearance of the dominant was accompanied by a reduction in the rate of accommodation to values below the initial level.

From these results we can postulate that the changes in the rate of nerve accommodation in the presence of a spinal cord dominant are also of an electrotonic nature; this fits in well with the ideas of the electronic nature of subordination effects [1, 2, 9, 11, 13, 14, 16, 17]. On the basis of the results of our experiments we can postulate that the spinal cord centers in the dominant state have catelectrotonic effects on the periphery, and the centers in a state of conjugate inhibition have anelectrotonic effects on the periphery. However, our earlier results [21] on the nature of the changes in motor nerve excitability on formation of a spinal cord dominant do not all fit in with this theory; as distinct from the case of catelectronus, the chronaxie is not increased, but reduced.

The reduction of the chronaxie of the motor nerve, according to published information [3, 4, 18], is associated with the subordination effect of the sympathetic nervous system. It is possible that this effect is also present on the formation of a spinal cord dominant. This hypothesis, however, requires further investigations.

SUMMARY

This work deals with a study of motor nerve accommodation following formation of a spinal cord dominant. The dominant was formed in one of the flexor centres of the posterior limb by acting upon the frog's sensory nerves with rhythmic subliminal stimuli. The presence of the subthreshold dominant was manifested in the reversal of the reciprocal relations between the antagonistic centers. As shown by experiments, the formation of a dominant in the spinal cord centres leads to an increased rate of motor nerve accommodation in the corresponding limb and its reduction in the contralateral limb. In their character and rapid reversibility these changes are very similar to those developing in the nerve in the area of catelectrotonus and anelectrotonus.

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THE EFFECT OF TEMPORARY EXCLUSION OF THE HEART FROM THE CIRCULATION ON THE HIGHER NERVOUS ACTIVITY OF DOGS IN A STATE OF HYPOTHERMIA

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The further development of cardiac surgery requires the urgent widening of the scope of possible surgical intervention in a "dry field" [3, 7, 13, 14]. Hypothermia is used for this purpose, as a method of increasing the resistance of the body and, in particular, of the central nervous system to temporary interruption of the blood flow [4, 5, 12]. The character of the functional changes developing in the cerebral cortex as a result of the temporary exclusion of the heart from the circulation under the conditions of hypothermia has not been discussed in the literature; its importance at the present time is obvious, for the study of this question will furnish more detailed and accurate information on the permissible periods of interruption of the circulation of the blood.

We have investigated the recovery of conditioned reflexes in dogs after exclusion of the heart from the circulation for 15 min during hypothermia.

METHODS

Experiments were carried out on 3 dogs. The animals had firmly established conditioned reflexes, produced experimentally and involving various analyzers of the cerebral cortex. The dog Sil'va had protective respiratory reflexes, reinforced by ammonia, to a bell, to a light and touch stimulus, with differentiation to a bell of another pitch. The dogs Mirnyi and Valetka had food conditioned reflexes (with a delay of 10 sec) to a metronome, a light and touch stimulus and differentiation to each in turn, the complete sequence forming a stereotype. Hypothermia was induced in the animals by means of a cold pack under general intubation anesthesia with ether and oxygen on a basis of morphine (0.5 ml/kg of 1% solution) until the rectal temperature was 24°. A right-sided thoracotomy was then performed and the heart excluded from the circulation by ligation of the azygos vein and clamping of the vena cava; fibrillation was prevented by the method of V. M. Pokrovskii and V. M. Bensman [10]. After restoration of the circulation the chest was closed without drainage and warming of the animals to a temperature of 32° commenced. The dogs were then taken from the operating table and subsequent experiments were carried out in the conditioned reflex chamber. We made 55 investigations of the conditioned reflexes in the three dogs after exclusion of the heart from the circulation on one occasion.

RESULTS

Immediately after its removal from the operating table the animal persistently held its head crown uppermost, reacted to a loud noise by turning its head, and made attempts to get up on to its legs, making haphazard movements with its paws. Tests of the protective respiratory reflexes in the dog Sil'va at this time showed that they were completely absent to all stimuli (see figure, a, b). Twelve hours after operation the dogs

TABLE 1.

Recovery of Conditioned Reflexes in the Dog Mirnyi after Exclusion of the Heart from the Circulation for 15 Minutes during Hypothermia

Conditioned stimulus	June 10, 1957 initial values		June 11, 1957		June 12, 1957		June 13, 1957		July 14, 1957	
			day after operation							
			first		second		third		fourth	
	latent period (sec)	magnitude of conditioned reflex*	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex
Metronome +240	3	9	—	0	12	2	—	0	—	0
Light	2	8	—	0	—	0	—	0	—	0
Metronome -60	3	4	—	0	3	6	—	0	9	2
Touch stimulus	9	3	—	0	—	0	—	0	—	0
Total		24		0		8		0		2

* The magnitude of the conditioned reflex is given in drops of saliva during the action of the conditioned stimulus alone for 30 seconds.

tried to walk; in so doing they staggered, collided with objects in their way and fell. The reaction to a summons by name was inconstant, and the animals did not recognize the experimenter. Conditioned reflexes were absent. After 24 hours the dogs walked with difficulty and staggered. The reaction to a summons was stabilized and a positive reaction to the experimenter also appeared. The animals, however, could not stand up on the bench, but hung on their straps. Tests of the conditioned protective reflexes in Sil'va showed them to be completely absent. Conditioned salivatory reflexes were found only in Valetka. Their magnitude in response to all positive stimuli was equal to one drop of saliva. The differential reaction was disinhibited to 5 drops. The higher response to the differential stimulus than to the positive led to a state closely resembling an ultraparadoxical phase in the cerebral cortex. This fact bore witness to the profound limiting inhibition of the cells of the cerebral cortex.

After 48 hours the animals' condition deteriorated. The body temperature reached 39-40°, and tachycardia and dyspnea were observed. Tests of the conditioned reflexes in the dog Sil'va were not carried out because of its poor general condition. The salivatory conditioned reflexes in Valetka were absent. In the dog Mirnyi, 2 drops of saliva were secreted in response to a strong stimulus--metronome; subsequent positive stimuli did not evoke reflexes, and the differential reaction was disinhibited to 6 drops.

After three days the conditioned reflexes were absent. The animals did not react to the offer of food, nor did they take food. An unconditioned reflex reaction to the odor of ammonia could now be observed in Sil'va. On the fourth day no respiratory conditioned reflexes were seen (see figure, c). Positive food reflexes were also absent; at this stage disinhibition of the differential reaction to 2 drops was observed in Mirnyi (Table 1).

Recovery of the respiratory conditioned reflexes began on the fifth day, when the conditioned reflex to the bell first appeared. Differentiation was retained at this stage (see figure, d). Tests of the salivatory conditioned reflexes in Valetka showed them to be absent to the metronome and light; meanwhile the effect of the weak touch stimulus was equal to the initial response (3 drops). The dog Mirnyi died on the fifth day from secondary hemorrhage from the femoral artery.

The next tests of the conditioned reflexes were made on the seventh day. The characteristic

TABLE 2.

Recovery of Conditioned Reflexes in the Dog Valetka after Exclusion of the Heart from the Circulation for 15 Minutes during Hypothermia

Conditioned stimulus	June 26, 1957		June 27, 1957		July 1, 1957		July 3, 1957		July 4, 1957		July 6, 1957		July 8, 1957		July 10, 1957		July 15, 1957	
	day after operation																	
	initial values		first		fifth		seventh		eighth		tenth		twelfth		fourteenth		nineteenth	
	latent period (sec)	magnitude of conditioned reflex*	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex	latent period (sec)	magnitude of conditioned reflex
Metronome +240 . . .	7	5	29	1		0	12	2	16	3	3	4	2	7	3	7	5	6
Light	9	3	4	1	–	0	–	0	–	0	–	0	–	0	8	3	7	4
Metronome -60. . . .	–	0	3	5	–	0	–	0	10	3	–	0	–	0	15	1	17	1
Touch stimulus	16	2	2	1	5	3	–	0	–	0	–	0	15	1	6	1	11	3
Total		10		8		3		2		6		4		8		12		14

* The magnitude of the conditioned reflex is given everywhere in drops of saliva during the action of the conditioned stimulus alone for 30 sec.

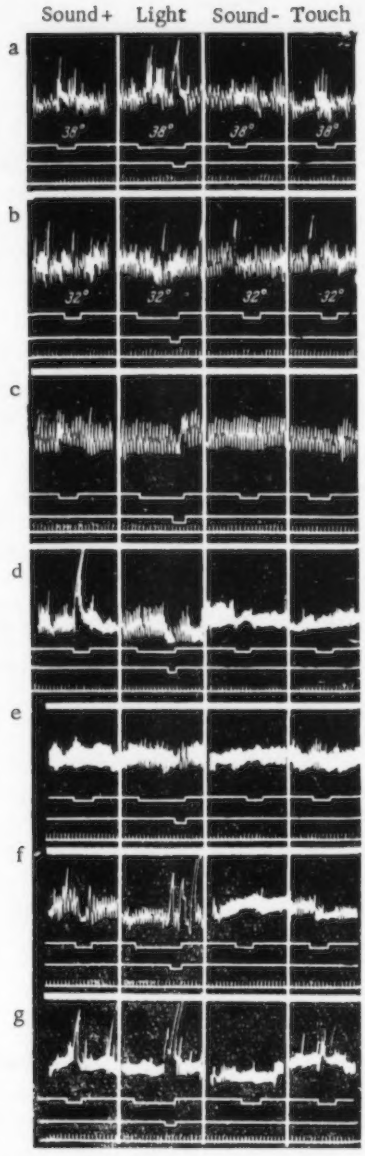

Fig. 1. Changes in the protective conditioned respiratory reflexes after exclusion of the heart from the circulation for 16. 5 min at a body temperature of 24°, in the dog Sil'va. a) Initial level of the reflexes; b) after exclusion of the heart, on the day of operation; c) on the 4th day after operation; d) on the 5th day; e) on the 7th day; f) on the 8th day; g) on the 10th day after operation. Significance of the curves (from above down): tracing of the respiration, application of the conditioned stimulus, application of the unconditioned stimulus, time marker (3 sec).

findings on that day were, that in Valetka a natural conditioned reflex to the sight and smell of meat-biscuit powder appeared simultaneously with the conditioned reflex to the metronome (2 drops). This was the first dog to take food from the basin after reinforcement of the conditioned stimulus. Respiratory reflexes were absent in Sil'va (see figure, e).

On the eighth day, together with the conditioned reflex to the bell, recovery of the conditioned reflex to light took place with well marked delay. The reaction to the touch stimulus was hardly perceptible, and differentiation was retained. Complete recovery of the protective respiratory reflexes took place on the tenth day (see figure f, g). Later investigations of the conditioned respiratory reflexes showed them to be normal.

Tests of the food conditioned reflexes in Valetka on the eighth day revealed a conditioned reflex to the metronome (3 drops of saliva). Disinhibition of differentiation to 3 drops was also observed at the same time. On the ninth day, investigation showed the absence of conditioned reflexes to all the stimuli in this dog.

During tests of the stereotype in Valetka nine days after operation a response reaction was observed only to the first strong stimulus—the metronome (4 drops of saliva). This can be regarded as an indication that the process of excitation in the cerebral cortex was rapidly exhausted, revealing the low limit of functional capacity of the cortical cells. Differentiation was not disinhibited. Starting at this time, the conditioned reflex to the metronome appeared persistently in all the subsequent experiments (Table 2).

On the 12th day, together with the conditioned reflex to the metronome a conditioned reflex to the touch stimulus appeared (7 drops and 1 drop of saliva respectively). This was the first animal to eat food after the operation with all forms of reinforcement. On the 14th day after operation artificial conditioned reflexes to all stimuli were found. Their magnitude, however, was not yet equal to their initial level; the latent period was appreciably shortened and the strength relationships between the individual stimuli were abnormal. Only on the 19th day did the conditioned salivatory reflexes revert to their initial values. The insignificant disinhibition of differentiation (1 drop) was evidence of some degree of weakness of the internal inhibition.

After exclusion of the heart from the circulation for 15 min in conditions of hypothermia, recovery of the conditioned protective respiratory reflexes thus began on the fifth day and was complete on the tenth day. Recovery of the salivatory conditioned reflexes after this operative procedure began on the seventh day and was complete on the 19th day after operation. In the course of recovery, all the animals showed transitional hypnotic phases, indicating the development of limiting protective inhibition in the cerebral cortex;

these were particularly noticeable during the first five days after operation. It should be pointed out that the process of recovery of the higher nervous activity showed a characteristic fluctuation, with the development of cycles of cortical activity; this also was evidence of the low limit of the functional capacity of the cortical cells.

Analysis of the rate of recovery of the different conditioned reflexes shows that it is dependent upon the type and strength of the conditioned stimuli. Quickest of all was the recovery of the conditioned reflexes from the auditory analyzer (bell, metronome), next—from the optic analyzer (light) and then—from the cutaneous analyzer (the touch stimulus). The rate of recovery is also dependent on the form of the reflexes; this is seen particularly clearly when the results obtained are compared with those of our previous experiments on the study of the effect of general anesthesia and hypothermia on the conditioned reflex activity of dogs. First to recover were the protective conditioned respiratory reflexes, and then the food reflexes [1, 9].

Test of the conditioned reflexes 2-3 months after exclusion of the heart from the circulation during hypothermia revealed no changes in the behavior and the character of the conditioned reflexes to artificial stimuli.

The interruption of the blood flow to the brain by exclusion of the heart from the circulation for 15 min during hypothermia thus causes well marked disturbances of the function of the cerebral cortex. These disturbances are reversible in character.

The production of cerebral anemia for this length of time (16 min) at the ordinary body temperature leads to irreversible changes in the cortical cells. Electrical defensive reflexes do not recover under these circumstances [11]. Normalization of the conditioned reflexes as a result of the subsequent exclusion of the anterior cerebral arteries (after an interval of 10-40 min) takes place on the 15th-19th day [6]. According to G. A. Akimov [2], the function of the cerebral cortex in man after arrest of the heart for 5-6 min at the ordinary body temperature is restored later than the activity of the lower divisions of the central nervous system; irritability and loss of memory are subsequently noticed. In patients who die, pathomorphological changes are found in the cells of the cerebral cortex.

The findings described show that the increased resistance of the cells of the cerebral cortex in dogs as a result of hypothermia leads to a prolongation of the period of exclusion of the heart from the circulation to 15 min. Under these circumstances the signal activity of the cerebral hemispheres is fully restored. The time of recovery of the conditioned reflex activity observed in our experiments agrees with that found by L. I. Murskii and V. I. Soboleva [8]. These authors showed that in animals surviving clinical death (for 30 min) under conditions of hypothermia, recovery of the higher nervous activity takes place within 13 to 23 days. In view of these findings it may be postulated that, in dogs, exclusion of the heart from the circulation for 15 min at a body temperature of 24-25° is not an upper limit. It is possible that this period may be extended to 20-25 minutes. A special study of this problem is required.

SUMMARY

The conditioned reflexes are completely restored after the exclusion of the heart from the circulation for a period of 15 min during hypothermia. Restoration of protective conditional respiratory reflexes begins on the 5th day and they normalize compltely on the 10th day. Normalization of the salivary conditioned reflexes starts on the 7th day and is completed by the 19th day. The process of restoration fluctuates with the development of hypnotic phases of cerebral cortex activity.

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RECOVERY OF CONDITIONED REFLEXES IN DOGS AFTER INDUCTION OF HYPOTHERMIA BY MEANS OF HEAD PACKS*

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The induction of hypothermia in animals by means of application of packs to the head causes an intensive fall in the temperatures of all divisions of the brain, and especially of the cerebral cortex. The body temperature under these conditions falls much more slowly [5, 6, 11]. In cats, when the temperature of the cerebral cortex falls to 14.7-18.7°, the rectal temperature is 28.8-32.2° [5], and in dogs, when the temperature of the cerebral cortex reaches 17-22°, the rectal temperature varies between 26 and 30° [6].

It has been shown that a low brain temperature increases the resistance of the central nervous system to anoxia [2, 4, 8, 9]. This enables the time of exclusion of the heart from the circulation during "dry field" operations to be extended. Meanwhile, when the animal is subjected to hypothermia by means of head packs, the body temperature does not fall to such low temperatures as lead to the development of ventricular fibrillation [5, 6]. Isolated cases of the clinical application of this method have been described [12].

The present investigation is concerned with the study of the restoration of the functions of the cerebral cortex after induction of hypothermia by means of head packs.

METHOD

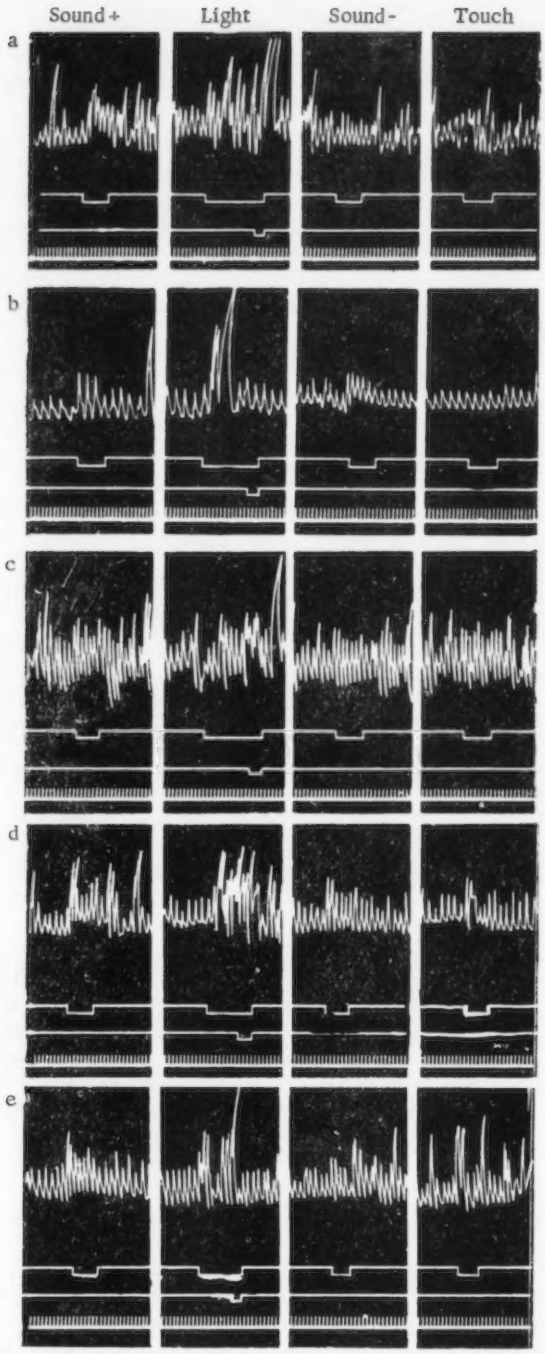
The investigation was carried out on two dogs. The dog Valetka had firmly established secretory food reflexes to a metronome, a light and a touch stimulus, and also one differential reaction. In the dog Sil'va protective conditioned respiratory reflexes had been established to reinforcement with ammonia. In order to secure the first action of cold on the brain, as a preliminary measure the temporal muscles had been removed from both dogs one year previously. Hypothermia was induced under morphine-ether-oxygen anesthesia. The animals' head was covered with a special double-layered cellular pack, through which cold water (3-5°) passed. When the rectal temperature was 30° the anesthesia was discontinued. When the rectal temperature reached 28° the induction of hypothermia was discontinued and warming of the trunk commenced by means of the passage of warm water (45°) through a cellular pack covering the animals' body. At a body temperature of 33° the warming was discontinued, the dogs were removed from the experimental table and further observations on the recovery of the conditioned reflexes were carried out in a conditioned reflex chamber.

On each dog 35 investigations of the conditioned reflexes were made after one induction of hypothermia.

RESULTS

The body temperature of the dogs reached 33° 1.5 hr after discontinuing hypothermia and starting to warm the animals. At this stage the dogs placed their head crown uppermost and carried out energetic movements of the head, and shivered. Stimulation by means of a weak rustling or tapping noise, or application of the conditioned stimuli caused intensification of the motor reactions. General behavioral reflexes to a summons by name or to the sight of the experimenter, and also the defensive reaction to threatening with a stick were absent in both dogs. The secretory food reflexes and protective conditioned respiratory reflexes, established experimentally in the animals, failed to appear.

*Given at a scientific meeting of the Kuban Medical Institute on May 14, 1959.



Recovery of protective respiratory conditioned reflexes in the dog Sil'va after hypothermia, induced by means of head packs, to a body temperature of 28°. a) Before hypothermia; b) on the 2nd day; c) on the 3rd day; d)on the 6th day; e) on the 7th day after hypothermia. Significance of the curves (from above down): respiration; marker of conditioned stimulus; marked of unconditioned stimulus; time marker (3 sec).

When the body temperature reached 34° the dogs tried to stand up but fell. Analysis of proprioceptive stimuli was disturbed. The unnatural position artificially assumed by the limb when flexed at the ankle joint was not corrected by the dog while the paw was involved in the general motor reaction. The natural and artificial conditioned reflexes were absent under these conditions (see table).

From 2.5-3 hr after discontinuation of the hypothermia, when the body temperature was 35°, the dogs were able to stand on their feet and to move about the laboratory. Nevertheless, their staggering and clumsiness of movement during walking were evidence of the incomplete recovery of coordination of movement. The shivering ceased. At this temperature the dogs first began to show general behavioral reflexes to a summons by name and to the sight of the experimenter.

An increase in the body temperature to 36° (4 hr after discontinuation of hypothermia) was not accompanied by any appreciable normalization of the conditioned reflex activity of the animals. Their behavior corresponded to that at a body temperature of 35°. Natural food conditioned reflexes to the sight and smell of meat-biscuit powder were absent in both dogs, and they did not take food. The protective conditioned respiratory reflexes were also not apparent.

On the second day after hypothermia, the dogs' body temperature was back at its initial level. The animals' behavior was not fully restored, and the dogs were lethargic and moved about little. The appearance of protective conditioned respiratory reflexes to the bell and light was observed in Sil'va (see figure, a and b). It can be seen from the figure that the conditioned reaction to the light was greater than the control magnitude of the reflex before hypothermia, whereas the conditioned reaction to the bell was greatly weakened. The protective conditioned respiratory reflex to a weak stimulus (touch) was absent. Differentiation was slightly disinhibited. The natural and artificial food conditioned reflexes were absent in Valetka (see Table). Under these circumstances, however, the recovery of the motor component of the food conditioned reaction was observed—in response to the conditioned stimulus the dog turned its head towards the feeding bowl but did not take food.

Recovery of the secretory food conditioned reflexes began on the third day after hypothermia. This was shown by the appearance of conditioned salivation in response to the metronome and light, and also of the natural conditioned reflex to meat-biscuit powder. The touch stimulus did not produce

Recovery of Conditioned Reflexes in the Dog Valetka after Induction of Hypothermia by Means of Head Packs to a Body Temperature of 28°

Date of experiment	11/XI	12/XI	13/XI	14/XI	15/XI	16/XI	17/XI	18/XI	19/XI	20/XI
	1958									
Body temperature	37.2°	34°	37°	37°	37.3°	37°	37°	37.2°	37.2°	37°
Metronome + 240	5	0	0	3	4	6	3	6	6	5
Light	4	0	0	1	4	1	1	1	4	4
Metronome – 60	0	0	0	0	2	1	1	0	0	0
Touch stimulus	3	0	0	0	2	1	1	2	2	3
Total	12	0	0	4	12	9	6	9	12	12

Note. The magnitude of the conditioned reflexes is given in drops of saliva during the action of the stimulus for 30 seconds. Hypothermia was induced on Nov. 12.

a salivatory conditioned reaction, and differentiation was not disinhibited (see table). It may be seen from the table that the total volume of saliva secreted in response to the conditioned stimuli throughout the whole experiment was one-third the initial value (4 drops instead of 12 of saliva). In Sil'va, on the third day after hypothermia, marked weakness of excitation was observed. This was shown by the low magnitude of the protective conditioned respiratory reflexes to the bell and light and by the complete absence of reaction to the touch stimulus (see figure, a and d). The dogs' behavior was normal.

On the fourth day after hypothermia, protective conditioned respiratory reflexes appeared in response to all stimuli, but were weaker than in the control tests. A similar picture was observed on the fifth day. Full normalization of the protective conditioned respiratory reflexes took place on the sixth day (see figure a, d, and e).

On the fourth day after hypothermia the strength of excitation in Valetka had perceptibly increased. Internal inhibition at this stage was severely weakened. In the experiment on November 15, 1958, for instance, shown in the table, the magnitude of the positive conditioned reflexes, characterizing the level of excitation, was almost the same as initially, whereas differentiation was disinhibited (2 drops).

On the fifth or sixth day after hypothermia the weakness of inhibition was less pronounced. Differentiation was disinhibited (1 drop of saliva). The magnitude of the conditioned reflex to the metronome reached its initial level (6 drops of saliva) on the fifth day after hypothermia. Meanwhile the limit of functional capacity of the cortical cells was severely reduced, and rapid exhaustion of the conditioned reaction developed. The first stimulus in the stereotype (metronome) evoked a much stronger effect (3-6 drops of saliva) than the later stimuli (1 drop of saliva).

On the seventh day after hypothermia internal inhibition was restored. Differentiation was complete, and a positive stimulus following it (touch stimulus) evoked a response a characteristic magnitude. The limit of functional capacity of the cortical cells was increased, although not to its initial level. This was confirmed by tests of the conditioned reflex to light (1 drop of saliva instead of 4) after a strong stimulus (the metronome).

Complete restoration of the secretory food conditioned reflexes took place on the eighth day after hypothermia (see table).

Investigation of the conditioned reflexes, like observation of the general behavior of the dogs during the following week and one or two months after hypothermia showed no abnormality in the conditioned reflex activity of the animals.

It must be pointed out that in Sil'va, on the day of induction of hypothermia, absence of the unconditioned protective respiratory reflex to ammonia was observed, and in Valetka the food reflex to meat-biscuit powder was absent. Complete recovery of the unconditioned protective respiratory reflex took place on the second day after hypothermia, and of the unconditioned salivatory reflex on the third day.

These facts show that the induction of hypothermia in dogs by means of head packs cuases a marked weakness of the fundamental nervous processes in the cerebral cortex. Excitation is disturbed to a lesser degree than internal inhibition. We discovered a similar pattern in 1957 after general hypothermia of the same dogs to a rectal temperature of 24° [1]. In this case, however, the recovery of the conditioned reflexes took place two or three times quicker than after induction of hypothermia by means of head packs. The explanation of this fact is that, by the latter method of cooling, the temperature of the cerebral cortex of the dogs falls much more rapidly than the rectal temperature, and the difference between then amounts to 8-10° [6, 11]. It may therefore be considered that after the induction of hypothermia in dogs by means of head packs to a rectal temperature of 28°, the temperature of the cerebral cortex fell to 18-20°, whereas after general hypothermia it fell to 24-26°. A direct relationship between the depth of hypothermia and the speed of recovery of the conditioned reflex activity has been established by O. A. Karpovich [3, 8]. In ther experiments on dogs subjected to general hypothermia to a body temperature of 25°, recovery of a motor conditioned reflex took place at 32°.

After cooling of the same animal to a body temperature of 28°, recovery of the motor conditioned reflex took place at a body temperature of 29°.

In conclusion it must be pointed out that the use of morphine-ether-oxygen anesthesia alone, without hypothermia, does not cause prolonged disturbances of the conditioned reflex activity. Under such circumstances the conditioned reflexes are restored during the first two days.

Meanwhile it has been found that the action potential of the brain (as shown by the electroencephalogram) during hypothermia disappear more rapidly the deeper the initial level of anesthesia [7, 10]. This demonstrates that anesthesia deepens the action of hypothermia, and may thereby lead to greater disturbance of the function of the cerebral cortex.

SUMMARY

Following the restoration of the body temperature in dogs, overcooled by head packs to the rectal temperature of 28°C, the secretory food and defensive respiratory conditioned reflexes are also completely restored. The process of restoration of the cerebral cortical function is 2-3 times slower than after external overcooling of the whole body. The restoration of defensive respiratory reflexes begins on the day after over cooling and is completed on the sixth day. The restoration of secretory food conditioned reflexes begins only on the third day after hypothermis and is completed on the 8th day.

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NEUROGENIC LESIONS OF THE MYOCARDIUM

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The fact has been widely reported in the literature that the myocardium may be damaged by the action of large doses of adrenalin [1, 6, 8]. We have previously reported [3, 4] that after a single intramuscular injection of a massive dose of adrenalin into rats (0.5-0.8 ml of a 1:1000 solution), significant changes in the ECG develop within a few hours, and at the end of 24-48 hr destructive histological changes appear in the myocardium.

The aim of the present investigation was to cause injury to the myocardium by a neurogenic method, i. e., by the action on the myocardium of adrenalin or some substance closely related to it by structure and mode of action, as an endogenous mediator liberated from the endings of postganglionic sympathetic fibres as a result of stimulation. For this purpose we applied excessive stimulation to the stellate ganglia, through which pass the main nerve connections of the heart.

METHOD

Experiments were carried out on 73 rats weighing from 250 to 350 g. Under light ether anesthesia a midline incision was made, the neurovascular bundle was opened up, and the stellate ganglion was dissected out under a loupe. The ganglion was seized between the blades of a hemostat for 5-15 min, after which the hemostat was removed, streptocide powder sprinkled into the wound and the skin sutured. No case of sepsis of the operation wound was observed. In one group of animals (13) trauma was applied to the left stellate ganglion, in another group (32) to the right, and in a third group (7) to both stellate ganglia. In 6 experiments the region of the stellate ganglion was liberally painted with turpentine and the would was then closed.

The control group consisted of 15 animals. Some of these remained intact, while in others the neurovascular bundle was opened under ether anethesia and the stellate ganglion dissected without subsequent trauma to it. The animals survived for periods varying from 4 hr to 11 days; 11 animals died at various intervals after the operation (from 4 hr to 7 days) and the rest were sacrificed by decapitation. The heart was extracted from the chest, rinsed with physiological saline, fixed in 12% formalin solution and embedded in paraffin wax. Sections were cut in a transverse direction at the level of the papillary muscles, and stained with hematoxylin-eosin, picrofuchsin (by Van Gieson's method) and, in some cases, with iron-hematoxylin (by Heidenhain's method). The ECG was recorded in some animals, using the three standard leads, at different times after operation.

RESULTS

The experiments showed that in 27 of 52 cases using mechanical trauma and in 4 of 6 cases using chemical trauma (painting the ganglion with turpentine) excessive stimulation of the stellate ganglia caused a neurogenic lesion of the myocardium, bearing the character of cloudy swelling. On microscopic investigation swelling

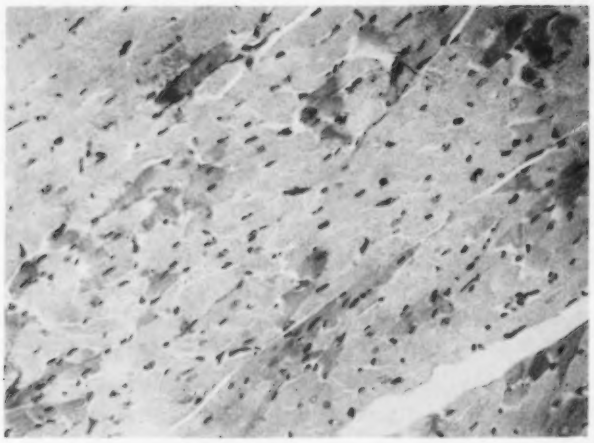

Fig. 1. Dystrophic changes in individual muscle fibers of the rat's heart. Trauma to the stellate ganglion. Stained by Van Gieson's method. Magnification 320 times.

Fig. 2. Destruction of muscle fibers with considerable cellular reaction. Trauma to the stellate ganglion. Stained by Van Gieson's method. Magnification 320 times.

of individual muscle fibers was found, with homogenization of the sarcoplasm and disappearance of the cross-striation. By comparison with the unchanged fibers, these were more darkly stained, so that in some cases the sections had a variegated appearance (Fig. 1). As a rule the manifestations of cloudy swelling were focal in character and affected only individual muscle fibers or groups of fibers, mainly in the left ventricle and the papillary muscles. Subsequently, depending on the time after infliction of the trauma, the patches of cloudy swelling either underwent resolution or, on the contrary, grew, leading to destruction of the muscle fibers and the formation of small foci of necrosis of the myocardium. The muscle fibers either melted away and dissolved (myolysis) or broke up into small fragments (plasmorrhexis). In the latter case a weak or moderately intensive cellular reaction was observed. Only occasionally (in 5 experiments) was considerable myocardial dystrophy observed, accompanied by intensive destruction of individual groups of muscle fibers and by well marked cellular infiltration (Fig. 2).

The blood vessels and capillaries were grossly dilated in some cases and filled with blood cells; sometimes small extravasations of blood from small vessels were seen. The ECG, taken at various times after operation, usually showed very slight voltage changes of the P, R, S, and T waves, with a small displacement of the ST interval from the isoelectric line.

Comparison of the results obtained after stimulation of the right, left or both stellate ganglia simultaneously showed no particular difference in the pattern of the myocardial lesion. For instance, of the 32 rats subjected to the trauma of the right stellate ganglion, dystrophic changes were observed in 18, and of the 13 rats in which trauma was applied to the left ganglion-in 4, and of the 7 rats in which both ganglia were injured-in 5. As regards the 6 cases of stimulation of the stellate ganglia with turpentine, dystrophic changes were observed in all three cases in which the left ganglion was treated and in one of the three cases in which the right was stimulated. The animals usually tolerated simultaneous bilateral trauma to the stellate ganglia and the application of turpentine to the region of the exposed ganglion very badly. Of the 7 animals undergoing operation, 5 died in the course of the first 24 hr in the first case, and 3 of the 6 in the second.

In order to induce experimental adrenalin myocarditis, most workers use the simultaneous injection of adrenalin and caffeine or theophyllin. It is considered that the latter increase the load on the myocardium and thereby predispose the heart to the development of destructive changes after the injection of toxic doses of adrenalin. In our experiments the subcutaneous injection of 1 ml of 10% caffeine solution at the same time as the trauma was applied to the stellate ganglia had no significant effect on the course or character of the cloudy swelling of the myocardium (19 experiments). Nor was any appreciable effect observed from the use of cortisone (20 mg/kg body weight, in a single dose or daily for one week), which, according to our experiments and reports in the literature [9], considerably aggravates lesions of the myocardium caused by adrenalin and noradrenalin (7 experiments).

In the discussion of the results obtained, the first question which arises is: to what extent may the changes in the myocardium brought about by a neurogenic mechanism be compared with the changes arising as a result of large doses of adrenalin or noradrenalin? Analysis of the findings showed that they have features in common. In adrenalin myocarditis, destruction of muscle fibers and the pronounced cellular reaction appear only at the end of the first 24 hours of the investigation. During the first few hours after the injection we observed nothing more than dystrophic changes in the myocardium: irregularity of staining, swelling, homogenization and fragmentation of individual muscle fibers, with loss of their cross-striation (Fig. 3), i. e., the same picture as in the

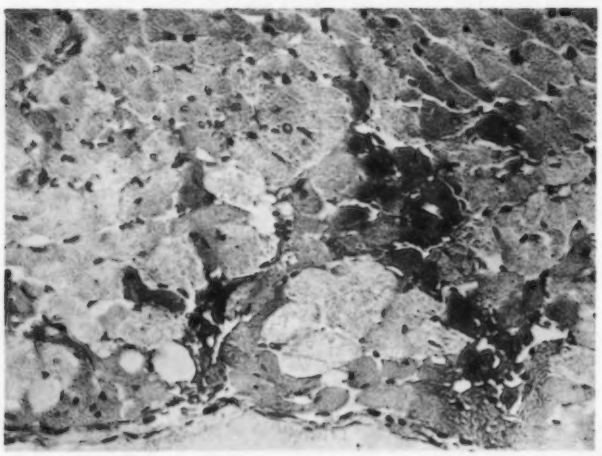

Fig. 3. Dystrophic changes in individual muscle fibers of the rat's heart 3 hours after intramuscular injection of 0.6 ml of 1:1000 adrenalin. Stained by Van Gieson's method. Magnification 320 times.

neurogenic lesions of the myocardium (see Fig. 1). After the action of toxic doses of adrenalin or noradrenalin on the myocardium, destruction of muscle fibers was more in evidence than dystrophy and was accompanied by intensive cellular infiltration; in neurogenic lesions of the myocardium the pathological process was more sluggish in its course and only in isolated cases was destruction of many muscle fibers an obvious cellular reaction (see Fig. 2).

In this case the picture of the myocardial lesion was little different from that caused by adrenalin. This gives grounds for the belief that in both cases the cause of the damage was the same: in one case adrenalin injected from an extrinsic source in a large dose, and in the other-adrenalin or some substance closely resembling it in structure and mode of action, liberated by the endings of the postganglionic sympathetic fibers during excitation.

If this is so, then a second question arises: what is the nature of the injurious action of adrenalin? Is it the result of the direct action of adrenalin on the adrenergic systems of the myocardial tissue, or is it primarily the result of changes in the blood supply of the myocardium and the consequent impairment of its nutrition [2], or is the principal role in the development of "adrenalin myocarditis" played by the nervous system and, in particular, by the functional state of the spinal centers of innervation of the heart [7]?

We are inclined to think that the destructive changes in the myocardium are the result of the action of adrenalin on the adrenergic systems of the myocardium. This hypothesis is supported by our experiments on the injection of large doses of pituitrin and nicotine. In these experiments we did not cause destructive changes in the myocardium, although the pressor effect was undoubtedly very pronounced, and the conditions were consequently right for the development of acute anoxia of the myocardium. Of the series of neurotropic drugs which we tested previously, affecting the reflex arc in different points (central, ganglionic and peripheral), only those possessing a well marked sympathicolytic and adrenolytic action were able to prevent the development of "adrenalin myocarditis"[3, 4]. Finally, the dystrophic changes arising in the myocardium after trauma to the stellate ganglia also support the view that a leading part is played by biochemical processes taking place in the adrenergic systems of the myocardium both during the injection of exogenous adrenalin in large doses and during the action of the endogenous adrenalin functioning as mediator.

SUMMARY

This investigation was aimed at obtaining myocardial lesions by a neurogenic route, i. e., by acting upon the myocardium with adrenalin and noradrenalin (mediators, appearing at the ends of postganglionic fibers of the cardiac sympathetic nerves after their stimulation). For this purpose very strong stimulation, either mechanical or chemical, was applied to the stellate ganglia. Myocardial lesions, of the character of cloudy swelling (swelling of individual muscular fibers homogenization of the sarcoplasm, disappearance of the cross-striation) appear after stimulation of the stellate ganglia. These phenomena of cloudy swelling are focal in character. Later, depending upon the stage of the disease, they either resolve or, on the contrary, are enhanced, leading to the formation of small foci of necrosis in the myocardium with mildly or moderately cellular reaction. Pronounced disintegration of separate groups of muscular fibers and considerable cellular reaction were recorded in individual cases.

Myocardial lesions, provoked by parenteral administration of massive doses of adrenalin, are compared to those resulting from the stimulation of the stellate ganglia, the mechanism of the action of these agents being discussed.

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ENZYMIC DECOMPOSITION OF THE CEREBROSIDES OF THE BRAIN

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The study of the cerebrosides of the brain has so far been confined to the more precise definition of their structure. The participation of the cerebrosides of the brain in the metabolism of that organ is a matter on which still little is known. In a survey paper presented to the First Symposium on Neurochemistry, Klenk [15] reported that in Gaucher's disease, Niemann-Pick's disease and certain other conditions there is an increase in the lipid content, and hence in the cerebroside content of the brain. An increase in the cerebroside content in the diseases mentioned above has also been described by Thannhauser [21]. We have observed a decrease in the quantity of cerebrosides in the brain tissue of rabbits in generalized tetanus, in gas gangrene and in strychnine poisoning[2]. These facts, together with a series of researches into the metabolism of the cerebrosides by means of C^{14}-glucose and C^{14}-galactose [4-7, 17-20, 23] suggest that these compounds possess definite metabolic activity. Meanwhile, in spite of numerous attempts, no research worker has yet succeeded in establishing the enzyme systems responsible for the reaction of decomposition of the cerebrosides in the brain of animals.

The first communication on this question was made in 1920 by B. I. Slovtsov [3]. He observed the breakdown of cerebrosides by emulsin—an aqueous extract of bitter almonds. On the other hand, many workers studying the cerebrosides have found no appreciable decomposition of these compounds by brain tissue. In 1921, for example, A. M. Georgievskaya [1] reported that even after prolonged autolysis, there was no decrease in the cerebroside content of brain tissue. In 1929, Jungmann and Kimmelstiel [13] claimed a slight but perceptible enzymic decomposition of the cerebrosides of the brain during autolysis, byt the results of their experiments are extremely contradictory and give no grounds for reaching a definite conclusion. We were unable to detect any appreciable decrease in the cerebroside content of a homogenate of rabbits' brain after autolysis for 24 hr at 37° [2]. All the data described above were obtained after autolysis and are open to justifiable criticism. In a well-known paper, however, Johnson, McNabb, and Rossiter [12] reported that after incubation of brain slices in buffer solutions of different pH for 10 days, no change in the cerebroside content was observed.

This unusual resistance of the cerebrosides to enzymic breakdown led Lumsden [16] and others to postulate that in ordinary conditions the enzymes which split up the cerebrosides are inactive, for they are surrounded by a monomolecular layer of anti-enzymes, produced by the oligodendroglia. In certain pathological conditions the oligodendrocytes are destroyed, creating the necessary conditions for action of the cerebroside-splitting enzymes. This hypothesis agrees to some extent with the findings of Thannhauser and Thannhauser and Reichel [22], who reported that they had observed the decomposition of cerebrosides by an extract of brain tissue, which took place only in the presence of large amounts of SH-groups. These authors suggest that this decomposition is associated with activation of an enzyme—cerebrosidase—by SH-groups.

The work of these authors just mentioned is, in fact, the only one in which mention is made of observation of the enzymic breakdown of the cerebrosides. We accordingly decided to repeat it in order to investigate the activity of the cerebrosidase in the brain of rabbits during those pathological processes which, as we previously showed [2], are accompanied by a decrease in the cerebroside content of the brain.

TABLE 1

Galactose Content of Cerebrosides in Rabbits' Brain Homogenate after Incubation (as % of dry proteins)

In cerebrosides					
free		combined		total	
with cystein	without cystein	with cystein	without cystein	with cystein	without cystein
2,39	2,29	0,81	0,88	3,20	3,17
2,53	2,60	0,92	1,00	3,45	3,60
2,35	2,36	1,06	0,88	3,41	3,24
2,31	2,51	0,93	1,12	3,24	3,69

TABLE 2

Analysis of the Incubation Mixture

pH of medium	Complete mixture		Without substrate		Without enzyme	
	with cystein	without cystein	with cystein	without cystein	with cystein	without cystein
Estimation of galactose in trichloroacetic filtrate as reducing substances (in ml thiosulfate)						
5.0	0,55	1.80	0.68	1,75	1.07	1.87
7.00	1.00	1.75	1.10	1.78	–	–
Estimation of galactose in trichloroacetic filtrate by anthrone method (in colorimeter readings)						
5.0	0.05	0.06	0.06	0.06	0.01	0.03
7.0	0.05	0.06	0.06	0.05	–	–
Estimation of galactose in hydrolyzate of precipitate by anthrone method (in colorimeter readings)						
5.0	0.15	0.15	0.08	0.04	0.11	0.12
7.0	0.15	0.13	0.05	0.05	–	–

METHOD AND RESULTS

We carried out the first experiments with homogenates of whole brain of rabbits. These were incubated for 48 hours at 37° in phosphate and phosphate-citrate buffers at pH = 7.3, in the presence of 200 mg of cystein. The substrate was the cerebrosides of the homogenate themselves. After incubation, the proteins and lipids were precipitated with trichloroacetic acid, the precipitate was removed by centrifugation, and the lipids were extracted in a Soxhlet apparatus successively for 12 hours each with acetone, ether and a 1:1 mixture of methanol and chloroform. After evaporation of the solvent, the lipids were hydrolyzed with 10% H_2SO_4, the mixture being heated on a boiling water bath for 2 hours. The cooled solution was filtered, and the cerebroside content of the filtrate was estimated as galactose by the anthrone method. Since brain tissue contains free and combined cerebrosides, we deemed it necessary to investigate both fractions.

It may be seen from Table 1 that the galactose content of the free and combined cerebrosides, expressed as a percentage of the dry proteins of the brain, in the samples with cystein was not different from that in samples without cystein. In both cases the content of cerebrosides was the same as in the brain of rabbits when determined immediately after death of the animals.

In the next series we fully reproduced the experiments described by Thannhauser and Reichel. The enzyme preparation was obtained from rabbits' brain. The brain of an animal which had just been killed was frozen in liquid air, ground to a powder and vacuum-dried at a low temperature. The 2 g of fine, dry powder thus obtained was extracted with 25 ml of 0.9% NaCl with constant agitation for 8 hours. The extract was centrifuged and the centrifugate dialyzed against distilled water for 48 hr, and after dialysis was used as the enzyme solution. According to Fujino [8], who repeated the experiments of Thannhauser and Reichel, this enzyme solution can be kept in the cold for several months without losing its activity. As substrate we used a 0.1% aqueous colloidal solution of pure cerebrosides, isolated from ox brain, as described in Fujino's paper [8].

The incubation mixture consisted of 1 ml of enzyme solution, 5 ml of substrate solution and 3 ml of buffer-veronal in the samples of pH 5 and 7, and citrate-phosphate in the samples of pH 8. To the test samples was added 1 ml of a solution of 20 mg of cystein, neutralized to pH 7. Incubation continued for 18-24 hours at 37°. During this time, according to the authors cited above, complete hydrolysis of the quantity of cerebrosides taken must have occurred. It was assumed here that each molecule of cerebrosides splits up into galactose and ceramide. Thannhauser and Fujino traced the rise in the galactose content of the incubation mixture by the increase in the content of reducing substances, which they determined by a modification of the method of Hagedorn and Jensen.

In the first experiments we determined the galactose content of the trichloroacetic filtrate after incubation as anthrone. No increase in the galactose was observed in any of the test samples. In the experimental conditions described, no breakdown of cerebrosides thus took place. This unexpected finding led us to carry out a series of experiments to determine the galactose content by the method of Hagedorn and Jensen. The results of this series of experiments showed that, in the samples containing cystein, the content of reducing substances was higher than in the samples not containing cystein. In Table 2 we give the results of a typical experiment; in this case, as in the experiments of Thannhauser and Reichel, the difference was greater at pH 5 and less at pH 7.

It was apparent that an increase in galactose content had taken place here, which must indicate decomposition of the cerebrosides. It follows from the figures in Table 2, however, that the content of reducing substances in the control samples, containing either enzyme without substrate or substrate without enzyme, in those cases in which cystein was added to them, was higher than in the samples not containing cystein. This difference in the samples containing enzyme alone, without substrate, was almost equal to that in the full test samples.

In the samples containing substrate but no enzyme the difference was significantly smaller.

Besides estimating the reducing substances, in the same experiments we made parallel determinations of galactose as anthrone, and we found no increase during incubation in any of the samples (see Table 2). The readings of the scale of the electrophotocolorimeter, shown in this table, correspond to the color given by the anthrone itself. As a control we determined the galactose in the hydrolyzate of the precipitate obtained by addition of trichloroacetic acid to the samples. In all cases the amount of galactose in the hydrolyzate of the complete samples was equal to that in the control samples not containing enzymes (see Table 2). The results described led us to postulate that the excess of reducing substances which Thannhauser and Reichel, and later Fujino, took to be an increase in galactose as a result of the breakdown of cerebrosides, was in fact due to the presence of cystein in the incubation mixture.

Our experimental results do not, however, suggest that the brain does not contain a cerebroside-splitting enzyme system, for if that were so it would be impossible to account for the fact, established by means of C^{14}-galactose and C^{14}-glucose, that the galactose in the cerebroside molecule may undergo metabolism. In our view, possible ways of investigation of these enzyme systems are shown by recently published work on the elucidation of the structural formula of the cerebrosides. These researchers set out to investigate if α- or β-galactose was a component part of the cerebrosides. For this purpose cerebrosides were subjected to the action of α- and β-galactosidases.

Hamasato [10] observed the breakdown of cerebrosides by β-galactosidase from the snail's liver, whereas α-galactosidase was inactive in his experiments. Kiss and Jurcsik [14] observed the breakdown of cerebrosides by an α-galactosidase obtained from emulsin. In 1956, Fujino and Negishi [9] published work in which they succeeded in splitting cerebrosides with digestive juices. These authors found that pancreatin, pepsin "b" and trypsin split off galactose from galactolipids. In the course of 2 hours 40% of the galactose is split off cerebrosides. All three preparations possessed both α- and β-galactosidase activity. Separation of these enzymes showed that only β-galactosidase possesses the property of splitting the galactolipids.

It will be seen from the papers cited that it is not yet fully clear whether α- or β-galactosidase splits galactose from the cerebroside molecule. On the other hand, nobody has isolated these enzymes from brain tissue, although the experimental results that have been described suggest that the enzymes which split the cerebrosides in the brain of animals belong to the galactosidase type.

SUMMARY

Until now all attempts to find a cerebroside-splitting enzyme system in the brain have remained unsuccessful. Thannhauser and Reichel observed the splitting of cerebrosides by a brain tissue extract in the presence of SH-groups. The reaction was evaluated by the rise in the amount of reducing substances in an incubated mixture. When reproducing the above experiments, the authors obtained negative results when galactose, split from cerebrosides, was determined by the specific reaction for sugar (with anthrone reagent). The cerebroside-splitting enzymes in the brain of animals probably belong to the galactosidase type.

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THE BIOPHYSICS OF EXCITABILITY

THE MEASUREMENT OF ADEQUATE SENSORY AND MOTOR STIMULATION IN MAN

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It was Lapicque who suggested the measure of excitability applicable to all excitable objects, which he called the "chronaxie." During the subsequent decades a wide range of investigations has been undertaken in various countries, some supporting, some criticizing and others extending the concept of chronaximetry. The last of these three subdivisions includes our own investigations. We have introduced the following amendments into the concept of the chronaxie.

1. The chronaxie is not lengthened during the relative refractory period of a nerve, as followed from Lapicque's theory, but on the contrary is shortened, with a considerable increase of the rheobase.

2. The cycle of recovery after each excitation is determined by the degree of mutual adequacy between the test stimulus and the functional condition of the biological substrate at the given moment. The recovery of excitability after excitation is characterized not by one single curve, but by a whole family of curves [3, 4, 6, 7]. Hence the concept of differential excitability arose.

3. Differential excitability shows itself as a selective reaction of cells to particular forms of natural or artificial stimulus in relation to the current functional state, and the more easily and clearly the resulting effect develops. Measurement of the energy of electrical stimuli and of stimuli adequate for the respective analyzers, applied to various objects, has shown [5, 6, 7] the presence of a minimum of threshold stimulating energy. This minimum, which has also been observed by other workers, does not correspond to the magnitude of the chronaxie and has an independent biological meaning. In order to determine the excitability by electrical stimuli and by stimuli adequate for the organs of the senses, and taking into consideration the minimum of stimulating energy, we measured in the first place the rheobase, and in the second place the threshold durations of the stimuli within the zone of the utilization time, i.e., at the level of 1.3 rheobases, within the zone of chronaxie, i.e., at the level of 2 rheobases, and at the level of 4 and 8-10 rheobases.

We know from the biophysical investigations of the excitability of different biological objects carried out by I. P. Shelkov [8], B. F. Verigo [1], Hoorweg [9], and others, that the curve of the relationship between strength of stimulation with an electric current and the time of its action is hyperbolic in character and may by expressed by the equation:

$$i = \frac{a}{t} + b,$$

where i is the threshold intensity of the current when in action for the time t; a and b are constants for a particular test-object and a given functional state.

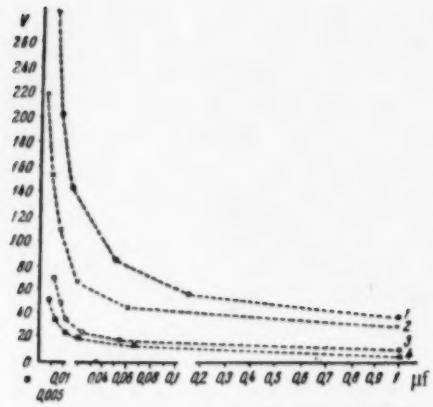

Fig. 1. Voltage–time curves. Along the axis of abscissas–capacity (in μ f) equivalent to duration of stimuli; along the axis of ordinates–threshold voltage of current; breaks in the axis of abscissas denote changes in the scale. 1) Thresholds for extensor digitorum communis; 2) threshold for flexor digitorum communis; 3) sensory thresholds in the extensor region; 4) sensory thresholds in the flexor region.

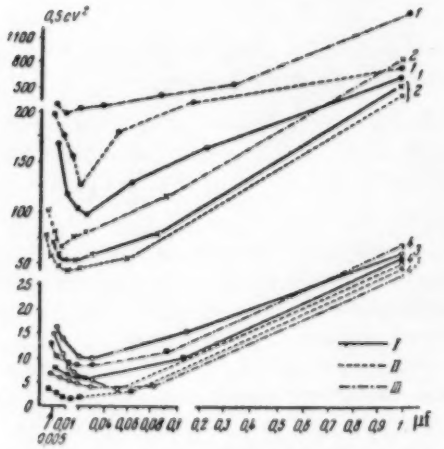

Fig. 2. Relationship between energy and duration of threshold stimuli. Along the axis of abscissas–capacity (in μ f) equivalent to duration of stimuli; along the axis of ordinates–energy of threshold stimuli–0.5 cV^2. I, II, III) results of testing of 3 different subjects. Notation for sensory and motor thresholds for flexor and extensor regions as in Fig. 1.

If a value of i is taken of twice the threshold value i = 2b, then,

$$i = \frac{a}{t} + b = 2b,$$

whence $2b = \frac{a}{t} + b$, or $2b - b = \frac{a}{t}$;

and consequently $b = \frac{a}{t}$.

From this equation the value of the second constant a = bt, and of the chronaxie–chr = $t = \frac{a}{b}$ is determined, which is usually done simultaneously from three zones of the strength–duration curve: 1) the zone of the utilization time at 1.3 rheobases, 2) the zone of relative adequacy with a minimum value of the threshold energy at 2-4 rheobases, and 3) the zone of the curve asymptotic to the axis of ordinates at 10-20 rheobases.

The object of the present investigation was to determine the laws of excitability: the strength–duration curve, taking into consideration the minimum value of the stimulating energy, i.e., measuring the relative thresholds of the extensor and flexor muscle groups of the upper limb in man, and their relationship to the excitability of the cutaneous receptor systems of the same area.

METHOD

In order to solve this problem we made use of the ordinary condenser chronaximetry with a Bourguignon shunt. The duration of the discharge of the condenser, of capacity 1 μf, was 4 msec, that of the 0.1 μf condenser was 0.4 msec and so on. Silver chloride electrodes were used. The method of stimulation was pseudo-unipolar. An indifferent electrode, 54 cm^2 in area, to the forearm, in the region of the motor points of the flexor and extensor digitorum communis, where the sensory and motor thresholds were determined. By changing the duration of the stimuli, we determined the threshold voltage of the current necessary to evoke a sensory and motor reaction, and we then constructed voltage–time curves and also curves showing the relationship between the energy of the threshold stimuli and their duration and intensity. The energy of the threshold stimuli E was calculated from the equation:

$$E = 0.5cV^2,$$

where c is the capacity of the condenser and V the voltage of the current by which it is charged.

The investigations were carried out on three healthy human subjects: 2 men–V. A., aged 26 years and E. G., aged 28 years, and one woman–T. K., aged 47 years. From 10 to 15 observations were made on each subject. The results obtained were treated statistically. We determined the arithmetic mean threshold value for stimuli of a given duration, and also the magnitude of the coefficient of variation.

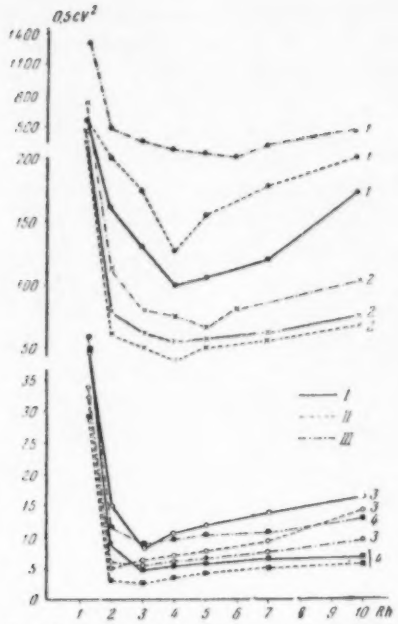

Fig. 3. Relationship between energy and intensity of subthreshold stimuli. I, II, III) results of testing of 3 different subjects.

RESULTS

In Fig. 1 a series of typical curves for the three subjects is given, showing the relationship between the voltage and duration of the threshold current (in Fig. 1 and in subsequent figures mean values are given—coefficient of variation within limits of 10 and 35%). It is clear from Fig. 1 that the voltage—time curves for both the sensory and motor effect have the usual hyperbolic appearance. Attention is drawn to the fact that the sensory thresholds are smaller for the flexor region than for the extensor. We observed this, however, only in the two subjects T. K. and V. A., and in the third, E. G., on the other hand, the thresholds for the extensor region were smaller than for the flexor. We may also mention that in all the subjects the difference between the values of the sensory thresholds for the flexor and extensor region increased as the length of the stimulus was decreased.

When the voltage—time curves for the motor and sensory effect obtained during stimulation of the same region are compared, it is clear that for all points on the curves the sensory thresholds are lower than the motor, and the difference between the sensory and motor thresholds is greater the shorter the duration of the test stimulus.

Comparison of the motor thresholds for the flexor digitorum communis and extensor digitorum communis shows that for the former it is less than for the latter, and in this case, moreover, the difference between the threshold values increases as the duration becomes shorter. Bourguignon's coefficient for the three subjects was 2-3 (3.3 in E. G., 2.5 in V. A., and 2.3 in T. K.), which agrees with data in the literature.

The curves given in Fig. 2 show the relationship between the energy and duration of threshold stimuli in all three subjects. We can see that the energy of the threshold current for both sensory and motor effect passes through a minimum at a definite duration of the stimulus. Comparison of the energy curves of the sensory effect obtained during stimulation of the flexor and extensor region corresponds to longer durations (0.03-0.7 μf) than that of the flexor region (0.02-0.03 μf). The same pattern is also observed for the motor thresholds; the minimum for the extensors corresponds to 0.014-0.034 μf and that for the flexors to 0.009-0.018 μf. We may also note that in all the three subjects the minimum zone of the sensory curves during stimulation of both flexor and extensor regions corresponds to a longer duration than the minimum zone of the curves obtained by recording the motor effect.

The relationship between the energy of threshold stimuli and their intensity, expressed in rheobases, is shown in Fig. 3. Results for all three subjects are given. It is clearly seen that, as the intensity of the stimulus increases, the curves pass through a minimum. For the majority of the curves the energy minimum falls at 3-4 rheobases (for the sensory effect at 2 and, more often, at 3 rheobases, and for the motor—at 4-5 rheobases).

These facts differ from those obtained by Lapicque, who claimed that the energy minimum is located at 2 rheobases. It may be seen from Fig. 3 that the parameters of a stimulus in the minimum zone (its intensity and duration) often do not correspond to the parameters of the chronaximetric stimulus.

SUMMARY

To characterize excitability the authors used the method suggested by P. O. Makarov elsewhere. It consists of determining the threshold duration of the stimulus at 3 zones of the strength-duration curve: a) at the utilization time zone, i. e., at the intensity level of 1—3 rheobases, b) at the minimal energy zone (at the level of 2—4 rheobases) and c) at the level of 8—10 rheobases. With the aid of condenser discharges the voltage-duration curves for the motor thresholds of flexors and extensors of the forearm and for the sensory thresholds of the corresponding skin regions were measured. In contradistinction to Lapicque's opinion, the minimum threshold energy is not always in proportion to the chronaxie duration of the stimulus, but corresponds to a rather wide region at the level of 2—5

rheobases. The minimal energy zone of the flexor muscles corresponds to shorter stimuli than that of the extensors. The difference between the sensory and motor thresholds for both flexor and extensor regions is the greater, the shorter the duration of the stimuli applied.

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THE EFFECT OF STROPHANTHIN ON THE CORONARY CIRCULATION AND CARDIAC OXYGEN INTAKE IN EXPERIMENTAL MYOCARDITIS

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Cardiac glycosides are extensively used to treat various diseases of the cardiovascular system often attended by coronary insufficiency. Despite the large number of works concerned with this question, however, the data describing the effect of cardiac glycosides on coronary circulation are rather inconsistent. Several authors, for example, assert that preparations of this group, used in doses the authors considered to approximate the therapeutic, either do not alter or somewhat increase the coronary blood flow [1, 7, 8-12, 18, 19, 25, 31, 37]. The works of other researchers have demonstrated that cardiac glycosides diminish the coronary blood flow [14, 16, 17, 33]. The majority of researchers concur in the fact that preparations of this group diminish the coronary blood flow when administered in toxic doses.

Research on the effect of cardiac glycosides on the oxygen intake of the heart muscle has produced data just as inconsistent as the results of the investigations studying the effect of these agents on the coronary circulation. It has been demonstrated on myocardial sections taken from different animals that cardiac glycosides in therapeutic doses increase the oxygen intake, but that when larger doses of these substances are administered, a considerable decrease in the oxygen intake occurs immediately after its increase [13, 15, 24, 29, 30, 35, 39]; it was further observed that the increase in oxygen intake was more pronounced on sections taken from a heart muscle poisoned with barbiturates [26]. In experiments with a contracting heart, it was demonstrated that strophanthin either does not effect the cardiac oxygen intake or increases it [7, 21, 23, 27, 28, 32, 34, 38], the latter being extremely pronounced if the oxygen intake was considerably reduced initially [35, 36]. The investigations of other authors, however, show the opposite, i.e., that strophanthin reduces the oxygen intake of the heart muscle [22, 33].

In this work, we studied the effect of therapeutic and toxic doses of strophanthin on the coronary circulation and cardiac oxygen intake of healthy animals and animals with experimental myocarditis. There are no data in the literature on the question of how cardiac glycosides affect the coronary circulation and the myocardial oxygen intake under conditions of experimental myocarditis, although this is a question of great importance to the more rational utilization of the given preparations.

METHODS AND RESULTS

Experiments were performed on cats (40 experiments) anesthetized with urethan and chloralose, and a few (eight experiments) were performed on healthy dogs. The change in coronary circulation was estimated according to the volumetric rate of the outflow of blood from the cat's coronary sinus by the method described in detail by N. V. Kaverina [5].

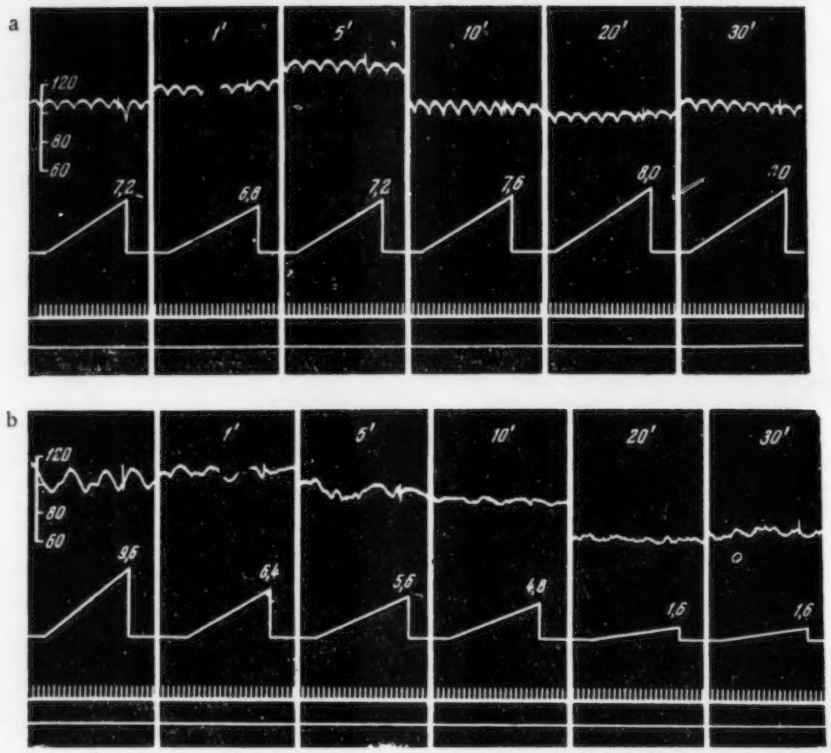

Fig. 1. Change in the volumetric rate of the coronary blood flow effected by strophanthin in doses of 20 μg/kg (a) and 50 μg/kg (b). Curves show (from top to bottom): blood pressure, volumetric rate of coronary blood flow (figures give volumetric rate of blood outflow in 1 min); time in seconds.

Oxyhemoglobin was determined in the blood flowing from the coronary sinus with the aid of an oxyhemometer. The cardiac oxygen intake was derived from the formula:

$$A = \frac{1{,}34\ (C_1 - C_2)\, V \cdot H}{100},$$

in which A is the amount of oxygen in ml/min, C_1, the oxyhemoglobin content of the arterial blood in %, C_2, the oxyhemoglobin content of the venous blood flowing out of the coronary sinus in %, V is the volumetric rate of outflow of blood from the coronary sinus in ml/min and N is the hemoglobin content in grams of 1 ml of blood.

The blood pressure was recorded in the carotid artery with a mercury manometer. Myocarditis was induced in the cats by the successive administration of theophylline (0.75 mg/kg) and adrenalin (1:1000 – 0.35 ml/kg). The presence of myocarditis was established by histology and electrocardiography.

The experiments showed that the effect of strophanthin on the coronary circulation and cardiac oxygen intake depends on the doses used and on the initial physiologic condition. For example, strophanthin in a dose of 10 μg/kg did not alter the volumetric rate of the coronary blood flow or the cardiac oxygen intake. In a dose of 20 μg/kg, strophanthin caused a slight increase in both the coronary blood flow and the cardiac oxygen intake (5-7%, see Fig. 1). In these doses, strophanthin had little effect on the heart rhythm or blood pressure; accelerated rhythm and slightly increased blood pressure were observed in a few experiments. In a dose of 30 μg/kg, strophanthin almost doubled the volumetric rate of the coronary blood flow. The increase in the coronary blood was attended by an increase in the myocardial oxygen intake, the latter increase being more pronounced (oxyhemoglobin content of the blood issuing from the coronary sinus was somewhat reduced). In a dose of 50 μg/kg, strophanthin caused a considerable decrease in the volumetric rate of the coronary blood flow (65%) and in the

cardiac oxygen intake (71%), a fall of blood pressure and the development of bradycardia. The decrease in the cardiac oxygen intake was usually more pronounced than that in the volumetric rate of the coronary blood flow (oxyhemoglobin content of the blood from the coronary sinus increased somewhat).

The coincidence of the changes effected by strophanthin in the volumetric rate of the coronary blood flow and the cardiac oxygen intake is physiologically wholly normal.

A different qualitative reaction was observed with the administration of strophanthin under conditions of experimental myocarditis (Fig. 2). In a dose of 10 μg/kg, strophanthin caused a considerable increase in the volumetric rate of the coronary blood flow (42%) and cardiac oxygen intake (39%).

In a dose of 20 μg/kg, strophanthin caused a considerable decrease in the volumetric rate of the coronary blood flow (43%) and the cardiac oxygen intake (50%). The decrease in the cardiac oxygen intake was stronger than that in the volumetric rate of the coronary blood flow, causing some increase in the oxygen content of the blood from the coronary sinus. Under conditions of experimental myocarditis, the decrease in the coronary blood flow induced by 20 μg/kg was attended by the development of bradycardia, which was not observed when these doses were administered to the healthy animals (Fig. 3).

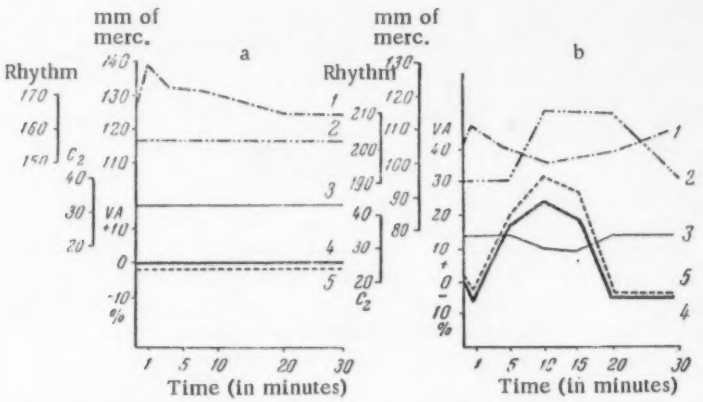

Fig. 2. Change in volumetric rate of coronary blood flow and cardiac oxygen intake effected by strophanthin in a dose of 10 μg/kg in normal animals (a) and in those with experimental myocarditis (b).
1) blood pressure; 2) rhythm; 3) oxyhemoglobin of blood of coronary sinus (C_2); 4) volumetric rate of coronary blood flow (V); 5) cardiac oxygen intake (A).

Under conditions of experimental myocarditis, the decrease effected by 20 μg/kg strophanthin in the volumetric rate of the coronary blood flow often did not fit the changes in the blood pressure and the frequency of the cardiac contractions. During the first minutes of strophanthin's action, for example, the blood pressure rose 10-20 mm of mercury and the heart rhythm either remained the same or became somewhat faster, but the volumetric rate of the coronary blood flow decreased considerably, even though one would expect an increased coronary blood flow under the given conditions.

Transection of the vagus nerves carried out on a background of the decrease in the volumetric rate of the coronary blood flow arrested this decrease, and some increase was even observed. After preliminary bilateral vagotomy, the decrease in the blood flow caused by strophanthin in a dose of 20 μg/kg was not as pronounced. For example, although strophanthin in this dose caused an average decrease of 43% in the blood flow, starting almost immediately after the administration of the preparation, after preliminary transection of the vagus nerves, the decrease in the volumetric rate of the coronary blood flow did not start until the 20th minute of the preparation's action and then constituted an average of only 9%; in some experiments, no decrease at all was observed—in fact, the blood flow increased somewhat during the early minutes of strophanthin's effect. After the preliminary administration of atropine in a dose of 1 mg/kg, no decrease was observed in the blood flow.

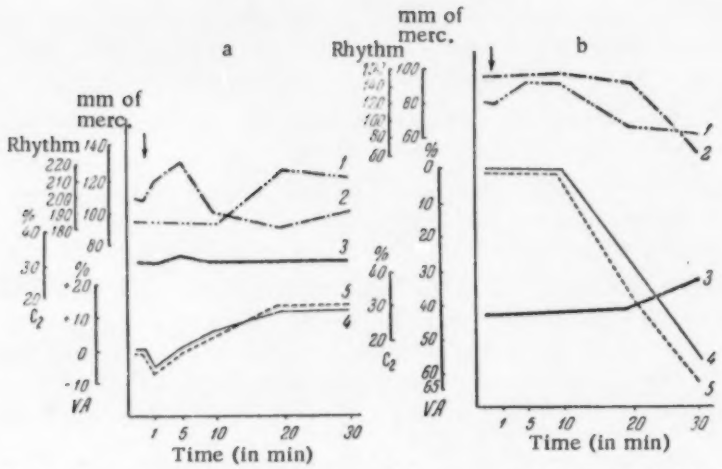

Fig. 3. Change in the volumetric rate of the coronary blood flow and in the cardiac oxygen intake effected by strophanthin in a dose of 20 μg/kg in normal animals (a) and in animals with experimental myocarditis (b). Curves the same as in Fig. 2.

Our study of the volumetric rate of the coronary blood flow and the cardiac oxygen intake of healthy dogs allowed us to establish that strophanthin in a dose of 30 μg/kg almost doubles the coronary blood flow and cardiac oxygen intake and that both these factors increase concurrently. In doses of 50-60 μg/kg, strophanthin decreases the coronary blood flow, cardiac oxygen intake and blood pressure and causes bradycardia.

We also conducted a series of experiments determining the toxicity of strophanthin according to the Wiengaarden method in healthy cats and in those with experimental myocarditis. It was established that strophanthin is considerably more toxic under conditions of experimental myocarditis. The average dose inducing cardiac arrest in healthy cats was 104 mg/kg* (with a variation of 0.095-0.111 mg/kg); under conditions of experimental myocarditis, this dose decreased to 0.76 mg/kg (with a variation of 0.050-0.097 mg/kg).

We also conducted investigations of cats to record the transmission of vagal excitation to the myocardium with continuous passage of a 1:200 000 strophanthin solution until cardiac arrest. We used both healthy animals and animals with experimentally induced myocarditis in the experiments. After transecting the vagal nerve, we stimulated its peripheral end with an electronic stimulator with a frequency of 30 hertz, each stimulus lasting 0.5 msec. The experiments showed that strophanthin considerably facilitates the transmission of stimulation from the vagus nerve to the myocardium under conditions of experimental myocarditis, but hinders the transmission of stimulation in healthy animals.

On the basis of the results obtained, one can conclude that strophanthin, administered in therapeutic doses to healthy animals, does not materially affect the coronary circulation of myocardial oxygen intake; usually, these indices either do not change or somewhat increase. In toxic doses, strophanthin causes the coronary blood flow, cardiac oxygen intake and blood pressure to decrease and bradycardia to develop.

The effect of strophanthin changes considerably under conditions of experimental myocarditis. In a dose of 10 μg/kg, strophanthin increases the volumetric rate of the coronary blood flow and the cardiac oxygen intake in cats with experimentally induced myocarditis. In the case of animals with experimental myocarditis, unlike that of healthy animals, strophanthin in a dose of 20 μg/kg diminishes the coronary blood flow and myocardial oxygen intake. This reaction is the most pronounced at the later periods in the development of myocarditis; on the seventh day of the development of myocarditis, strophanthin administered in these doses even caused death in some cases. There is a greater decrease under these conditions in the myocardial oxygen intake than in the volumetric rate of the coronary blood flow, which is typical of stimulation of the vagus nerves [20].

With experimental myocarditis, strophanthin in a dose of 20 μg/kg not only decreases the coronary blood flow and cardiac oxygen intake, but also induces bradycardia, which does not occur in healthy animals given these doses.

*Value given is as it appears in original Russian article. Value should probably be 1.04 mg/kg.

The different qualitative reaction to strophanthin exhibited by the animals with experimental myocarditis may be partly due to the increased sensitivity of a heart in this condition to cardiac glycosides. The increased toxicity of cardiac glycosides in experimental myocarditis has been mentioned in several works [2, 3, 6]. There is also the fact that strophanthin hinders the transmission of stimulation from the vagus nerves to the myocardium in healthy animals, but considerably facilitates it under conditions of experimental myocarditis [4].

The influence of the vagus nerves on the development of strophanthin's effect is further confirmed by the fact that the decrease in the coronary blood flow effected by these doses of the preparation in experimental myocarditis is only slightly apparent after transection of the vagus nerves, while transection of the vagus nerves carried out on a background of decrease in the coronary blood flow effected by strophanthin causes the blood flow to increase. In similar investigations with preliminary vagotomy, no decrease in the coronary blood flow was observed with the action of cardiac glycosides [17].

However, it is difficult to say whether the decrease in the coronary blood flow is due to the direct influence of the vagus nerves on the coronary vessels or is caused by changes in the heart action or metabolic processes which occur during stimulation of the vagus nerves.

The data indicating the increased toxicity of strophanthin in experimental myocarditis and the decrease in the coronary blood flow effected by doses which do not materially affect the volumetric rate of the coronary blood flow in healthy animals should be taken into account by clinicians.

SUMMARY

Strophanthin given in therapeutic doses to healthy animals has no significant effect upon the coronary circulation and oxygen intake by the cardiac muscle. Toxic doses reduce the coronary circulation, the cardiac oxygen intake and the blood pressure; bradycardia appears. The dose of 20 μg/kg, which causes no changes in the coronary blood flow or the cardiac oxygen intake in the healthy animals, considerably reduces these values in animals with experimentally induced myocarditis. The different qualitative reactions occurring in response to strophanthin administration to animals with experimentally induced myocarditis may be partially explained by the increased cardiac sensitivity to strophanthin in this condition and the intensified excitation of the vagus nerves; the reflex character of the latter effect follows from experiments with vagotomy.

The data on the increase in strophanthin toxicity and the different qualitative reaction of the coronary circulation observed in experimental myocarditis should be taken into consideration by clinicians treating diseases of the cardio vascular system with cardiac glycosides.

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THE EFFECT OF SODIUM NUCLEATE ON THE COURSE AND ISSUE OF LEUKOPENIA INDUCED BY TETRATHIONE (TETRAMETHYLTHIURAM DISULFIDE)

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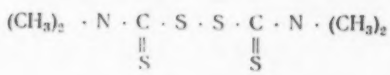
In the past few years, the arsenal of therapeutic agents available for the treatment of leukoses has grown considerably (nitrous analogs of yperite, AFK* myelosan (1, 4-dimethanosulfonylhydroxybutane), 6-mercaptopurine, ethylenimines, et al.). Nevertheless, the question of the treatment of leukoses is not yet solved. Research in this field, therefore, continues.

As we have already demonstrated, sulfides of the thiuram group (Antabuse, Tetrathione and sodium dimethyldithiocarbamate) act to inhibit leukopoiesis. Tetrathione (tetramethylthiuram disulfide, TMTD):



is the most active in this respect.

TMTD is a powder insoluble in water. It has long been used by industry to vulcanize resin [8]. Agriculture is now beginning to use it extensively as a fungicide, seed-dip and antioxidant in the storage of products [2, 3, 9-12].

The lethal dose of TMTD (introduced into the stomach once) is 700 mg/kg for rats and 1250 mg/kg for guinea pigs [1, 4, 5]. We have established that TMTD, introduced in a dose of 40 mg/kg or more repeatedly into the stomach, usually induces in rabbits leukopenia of the agranulocytic type which is not spontaneously resolved. Leukopenia develops as the result of the inhibition caused by the preparation of the myeloblastic hemopoietic function. In an absolute majority of animals, the hemoglobin and number of erythrocytes increase, as does the number of erythroblastic forms in the bone marrow [4, 5].

In this work, we studied the effect of sodium nucleate on the course and issue of leukopenia induced by TMTD. This is a question of real interest in view of the irreversible nature of such leukopenia, the introduction of TMTD into agriculture (where accidental poisoning is possible) and the fact that it has been recommended that the drug be clinically tested.

METHOD

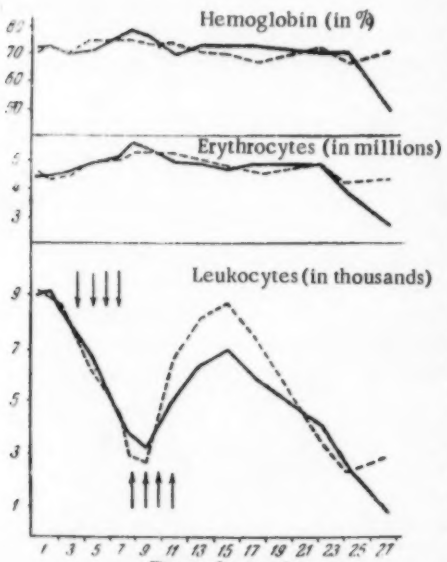
The experiments were performed on 15 male rabbits weighing 1.9-3.5 kg. After establishing the original "background" of the peripheral blood, we introduced TMTD (suspension in a 2% starch paste) in a dose of 40 mg/kg into the stomach of the animals once a day for four successive days in order to induce leukopenia.

*AFK is transliteration of Russian.

Arithmetic Mean Indices of Hemoglobin, Erythrocytes, Absolute Number of Lymphocytes and Pseudoeosinophils in the Rabbits' Blood

Day of experiment	Control				Experiment			
	hemoglobin (in %)	erythrocytes (in millions)	lymphocytes	segmented pseudoeosinophils	hemoglobin (in %)	erthrocytes (in millions)	lymphocytes	segmented pseudoeosinophils
1 st	72	4 671	6 397	2 420	71	4 500	6 002	2 671
2 nd	72	4 364	6 518	2 412	73	4 492	5 903	2 831
3 rd	71	4 554	6 258	2 175	70	4 592	5 298	2 830
5 th	75	4 998	4 977	1 409	71	4 995	4 763	1 797
7 th	75	5 085	4 308	961	77	5 367	3 122	1 547
8 th	75	5 337	2 408	534	79	5 724	2 485	642
9 th	74	5 324	2 146	521	77	5 524	2 746	565
11 th	74	5 321	4 802	1 588	70	5 078	3 859	1 116
13 th	71	5 000	4 935	2 952	73	5 000	4 183	3 467
15 th	70	4 890	4 483	3 933	73	4 767	3 698	2 956
17 th	67	4 671	4 510	2 778	73	4 900	3 797	1 950
22 nd	72	5 096	3 017	942	71	4 951	3 292	1 931
24 th	67	4 252	2 377	569	71	3 868	2 286	376
27 th	72	4 330	—	—	49	2 652	—	—

Note. TMTD was administered on the 4th, 5th, 6th, and 7th days, sodium nucleate on the 8th, 9th, 10th, and 11th days.



Dynamics of change in hemoglobin, erythrocytes and leukocytes in rabbits with leukopenia treated with sodium nucleate (average data). Key: ——) experiment; - - -) control; ↓) days of tetramethylthiuram disulfide administration; ↑) days of sodium nucleate administration.

Twenty-four hours after the fourth administration of TMTD, when leukopenia had developed in the animals, the rabbits were divided into two groups. The first, or experimental, group (eight rabbits) received sodium nucleate subcutaneously in a dose of 30 mg/kg once a day for four days in succession; the second, or control, group (seven rabbits) were subcutaneously injected with distilled water. First to establish the "background" and then during the development of leukopenia, the peripheral blood was examined every day for three days, after which it was examined every other day in most cases. We determined the hemoglobin, erythrocytes, total number of leukocytes, leukocyte formula and absolute number of lymphocytes and segmented pseudoeosinophils in the peripheral blood. Punctates were taken twice (during leukopenia and after treatment with sodium nucleate) from the epiphysis of the animals' tibia. We determined the total number of nucleated cells (myelokaryocytes) in the bone marrow. The customary experimental and clinical methods were used to examine the peripheral blood and the bone marrow. The efficiency of sodium nucleate was gauged according to the increase in the total number of leukocytes and myelokaryocytes, the normalization of the blood formula and the times at which the animals died in the control and in the experiment. The results of the experiments were recorded, and the average data were then computed from them. The table and the figure show average data from observations on seven experimental and seven control rabbits.

RESULTS

The figure shows that the total number of leukocytes in the blood of the experimental rabbits decreased, after the fourth administration of TMTD, from 8954 (initial norm) to 3171 per 1 mm^3 (eighth day of experiment). The absolute number of lymphocytes decreased 2.3 times (from 5734 to 2485), and the segmented pseudoeosinophils decreased 4.3 times (from 2777 to 642).

Treatment with sodium nucleate was undertaken on this background. After four injections of the leukopoiesis stimulator, the number of leukocytes in the rabbits had increased on the 15th day to 7000 per 1 mm^3 (see figure), the increase consisting mostly in granulocytes. Subsequently, in spite of the treatment administered, the number of leukocytes began to decrease gradually, constituting 925 per 1 mm^3 on the day of the last blood examination. A sharp decrease in the number of granulocytes was observed during this period in all the rabbits.

The leukocytes of the control rabbits showed similar changes, although the increase in their number (succeeding the primary leukopenia) was more pronounced (see table and figure). TMTD caused the total number of leukocytes in the blood of the control rabbits to decrease from the original norm of 9020 to an average of 2992 per 1 mm^3. Then, after spontaneously increasing to 8764 (on the 15th day of the experiment), their number gradually decreased, constituting 3066 per 1 mm^3 on the last day of the investigation (see figure). As in the experiment, the granular leukocytes were largely responsible for these changes (see table).

The data obtained indicate that sodium nucleate does not promote an increase in the number of leukocytes in the blood of rabbits with experimentally induced "Tetrathione" leukopenia. This was further confirmed by the results of the bone marrow investigations.

For example, we counted an average of 35,830 myelokaryocytes per 1 mm^3 of bone marrow from the experimental rabbits during the leukopenia period, and an average of 41,520 after treatment with sodium nucleate. The number of myelokaryocytes counted in the control animals during these same two periods of the investigation was respectively 32,820 and 50,640 per 1 mm^3. If the final results are disregarded, no differences were observed in the dynamics of the erythrocytes and hemoglobin in the experimental and control rabbits.

As the table shows, the administration of TMTD in both cases was attended by an increase in the number of erythrocytes and amount of hemoglobin per unit of blood volume. At the end of the experiments, we observed a decrease in the hemoglobin and erythrocytes of four out of seven experimental rabbits, and this decrease, naturally, was reflected in the average indices; in the control group, however, we only observed a slight increase in the amount of hemoglobin and number of erythrocytes in two rabbits, which did not affect the character of the average data.

All the control and experimental animals died. One rabbit of the experimental group died after the second injection of the leukopoiesis stimulator (data obtained from this animal were not in computation of the averages). 12 rabbits died between the 24th and 27th days of the experiment and two (one experimental and one control) died two months after the observations commenced.

The investigations described allow one to assume that sodium nucleate, subcutaneously administered in a dose of 30 mg/kg daily for four consecutive days does not eliminate leukopenia caused by TMTD. Indeed, the decrease in the erythrocytes and hemoglobin and the smaller increase in the leukocytes and myelokaryocytes observed in the experimental rabbits compared with the control would seem to indicate that sodium nucleate has a negative effect on hemopoiesis when administered on a background of the action of TMTD. TMTD leukopenia can be used as a "model" in the hunt for new stimulators of leukopoiesis.

SUMMARY

Tetrathione introduced into the stomach of rabbits daily for four consecutive days, in a dose of 40 mg/kg, provokes a granulocytic type of leukopenia which fails to disappear spontaneously. Sodium nucleate, injected subcutaneously for four consecutive days, once daily, in a dose of 30 mg/kg, does not improve the state of leukopenia provoked by Tetrathione.

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SECONDARY IMMUNOLOGICAL REACTIVITY IN INVERTEBRATE ANIMALS

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(Presented by Active Member AMN SSSR, N. G. Khlopin)
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The possibility of reproduction of immunological reactions in lower animals is not without grounds, for we know that various forms of immunity, like infectious diseases, are encountered at all levels of the zoological scale. A. A. Bogomolets [1] successfully immunized a mussel (Mytilus galloprovincialis) by injection of cultures of Escherichia coli. Ishimori [2, 3] and other authors have reported successful experimental immunization of insect larvae with Galleria melonella, a species of bacteria pathogenic towards them. Several papers indicate the possibility of the production of immunity in members of the phylum Arthropoda.

It is of practical importance to obtain a firm answer to the question of the secondary immunological reactivity of invertebrate animals, for the lower animals may be a very convenient object for the study of certain general problems of immunobiology.

METHOD AND RESULTS

The research into secondary immunological reactivity in invertebrates, which we carried out from a base at the White Sea Biological Station of Moscow State University, was of the character of a preliminary investigation. In the first place a number of technical problems had to be solved: the choice of experimental animals, the method of preparation of the antigen, the mode of immunization and dose of antigen injected, and the choice of reactions to be used for determining the immunological response in the recipients. Initially for the immunization experiments we used four phyla of marine animals, occupying close but different levels on the biological scale: the Chordata, Mollusca, Echinodermata and Annulata. From the phylum of the Chordata, 4 species of ascidia were used in the experiments—Styela rustica, Molgula retortiformis, Hyalocenthia pyriformis and Boltenia echinata; from the phylum of the Mollusca one species was studied—Mya arenaria; from the Echinodermata—Asterias rubens; from the Annulata—2 species, Nereis pelagica and Arenicula marina. Antigens for immunization of the animals were prepared from the tissues of ascidia and molluscs. For control and cross-testing purposes antigens were used from the tissues of all the phyla and species of animals listed above.

Antigen was prepared in the form of a tissue extract in sea water and, in a parallel series, in sea water with physiological saline (0.6% NaCl). The antigen was administered (in a dose of 0.3-0.5 ml per animal) by two methods: by injection with a syringe (0.5 ml) or in sterile glass capillary tubes (0.3 ml). Since the worms died 24-48 hours after receiving the foreign protein and sea water, they were not used in the subsequent experiments, and the "antigen" prepared from the tissues of the Annulata was used only in the performance of control reactions.

The experimental animals were kept in natural or close to natural conditions: molluscs in soil; littoral animals, ascidia—in a tank, kept in the sea; the starfishes in an aquarium with frequently changed sea water. In

Results of the Ring-Precipitation Test Between the Celomic Fluid of Molluscs Immunized with Tissue Proteins of Ascidia ("Antibodies") and Tissue Extract of Ascidia ("Antigens")

Series of experiments	Celomic fluid of molluscs immunized by single or repeated injections of antigen (0.3 ml of tissue extract each injection)	Result of investigation of "antibodies" in celomic fluid of molluscs, taken at different times after immunization of animals	
		after 24 hours	after 7 days
First	Immunization of animals with one injection of antigen	–	+
Second	Daily injection of antigen on 3 consecutive days	–	+
Third	Daily injection of antigen on 5 consecutive days	–	+
Fourth	Molluscs kept continuously in "ascidium" medium for 4 days (oral immunization).	–	–
Fifth	Continuous oral immunization for 4 days and one injection of antigen from syringe	–	+

Note. The sign – denotes a negative reaction, + a positive reaction

order to determine the presence or absence of an immunological response in the recipients, the ring-precipitation test and the precipitation test in agar capillary tubes were used. The animals to be immunized were sacrificed and investigated at different periods: from 2 to 30 days after immunization. The celomic fluid of the animals was tested with tissue proteins (antigens) of various species of invertebrate animals – members of the four above-mentioned phyla.

In the preparation of the antigen all the soft tissues of the animal were usod (usually pooled tissues from 5-10 animals of the same species were taken together; tissue "antigens" of the Chordata were prepared from a mixture of the organs of the 4 species of ascidia). The tissues taken were washed once with sterile sea water, minced with scissors and then thoroughly ground in a porcelain mortar. The minced tissue was mixed with an equal volume of sea water, the mixture centrifuged for 40-50 min at 2000 rpm and the supernatant fluid was investigated. The celomic fluid of the immunized animals, to be tested for the presence of precipitins, was extracted as follows: from the body of the ascidia – with a Pasteur pipette (after preliminary removal of sea water from the animal's stomach), and from the molluscs and starfish by thorough mincing and expression of fluid from the tissues of the animal. The fluid obtained in this manner contained, besides tissue proteins and juices, cells of different organs and sea water. The fluid was centrifuged and its supernatant portion was investigated – whole, or diluted 1:2 and 1:4. A disadvantage of this method of obtaining fluid is that the dose of "antibodies" used in the tests cannot be determined or measured. It is obvious that this method can only be used when the question to be solved is merely whether a secondary immunological reaction can be reproduced in invertebrates, and also provided that the experimental conditions include the frequent repetition of the tests and the use of large numbers of animals.

Observations on animals of the phyla Chordata and Echinodermata, immunized with the tissue proteins of molluscs, gave negative results in all series of our experiments.

We paid special attention to the study of the immunological reaction of the Mollusca after administration of proteins from the Chordata, i.e., animals with a more complex biological organization. We immunized altogether 63 molluscs with extracts of tissues of the Chordata. The results of these experiments showed that the celomic fluid of molluscs, immunized with tissue proteins of the Chordata, gave a positive precipitation test with the proteins of these animals in the majority of cases investigated – in 48 of 63, and at times between the 10th and 29th days. It was also found that this reaction was clearest in cases when the antigen was prepared in sea water without the addition of physiological saline and when the tissue extract was given to the animal by direct injection from a syringe and not in capillary tubes.

Occasionally the celomic fluid of control (unimmunized) molluscs also gave a positive precipitation test with tissue proteins of the Chordata. In these cases, however, the precipitate was formed only 40-60 min after

setting up the test, and moreover the precipitation ring had a tendency to be displaced upward—towards the celomic fluid. So far as the other control tests were concerned—the reaction between the tissue proteins of the Chordata and the celomic fluid of the Mollusca, immunized with the tissue proteins of other Mollusca or of animals of another phylum—in all these cases they were negative.

The preliminary findings obtained in this research led us to conclude that the experiments in which Mollusca were immunized with tissue proteins of the Chordata must be repeated.

For preparation of the "antigen" the tissues of three species of ascidia were used: Styela rustica, Molgula retortiformis and Boltenia echinata (10-20 animals of each species). The molluscs were immunized by means of injection of tissue extract with a syringe (0.3 ml at each injection).

The response reaction of the molluscs was tested at various times—from one to seven days after the last injection of tissue extract.

The experimental animals were divided into four groups with 40 animals in each. The animals of the first group were immunized once. Half the total number of animals in the group were sacrificed and tested 24 hr after immunization and the other half 7 days after immunization. The animals of the second group were immunized three days in succession and investigated at the same intervals (24 hr and 7 days after the last injection of antigen). The animals of the third group were immunized five days in succession and also investigated after two intervals. The animals of the fourth group were immunized per os: they were kept in an aquarium with sea water, to which was added every day a mixture of minced tissues of ascidia of the three species mentioned above (100 mg of tissue to 5 liters of sea water). The medium was enriched with oxygen by the periodic pumping of air into the "ascidium" medium. One part of these animals was investigated immediately after continuous oral immunization for four days, and the rest 24 hours after supplementary immunization of the animals by injection of antigen from a syringe.

In order to study the immunological response reaction in the experimental molluscs, at suitable times the celomic fluid was extracted and investigated by the ring-precipitation test with "antigens" prepared from ascidia tissues. The celomic fluid and "antigen" were used in the test undiluted and in dilutions of 1:10, 1:100 and 1:1000.

The results of the investigation of the "antibodies" in the celomic fluid of the immunized molluscs are summarized in the table.

It will be seen from the results described that the ring-precipitation test was positive in by no means every case. In not one series of experiments when the celomic fluid of the experimental molluscs was tested 24 hr after immunization of the animals could the presence of precipitins be demonstrated in this fluid. The precipitation test was positive only in those cases in which the celomic fluid of the immunized molluscs was tested 7-12 days after the first injection of antigen. Oral immunization of the molluscs did not give positive results when the celomic fluid of the animals was tested on either the first or seventh day after immunization.

It can thus be concluded from the results of these experiments that invertebrate animals of the phylum Mollusca react to injections of foreign protein (tissue protein of invertebrates of the phylum Chordata, i.e., animals at a higher level in the scale of biological organization) by the production of precipitins, which can only be demonstrated after a certain time has elapsed since immunization of the animals. The number of injections of "antigen," as the experiments showed, did not play a decisive part in the formation of "antibodies" in the experimental animals. Consequently, the formation of precipitins (the possibility of detection of precipitins in the celomic fluid) in the experimental animals depends not so much on the dose of antigen injected as on the time elapsing between the day of immunization and the day of investigation of the celomic fluid.

On account of the short duration of our expedition we were unable to find out how long the precipitins remained detectable in the celomic fluid of the immunized molluscs. We can only state that the reproducibility of the findings affords convincing evidence of a regular pattern, the nature of which cannot yet be explained. We also find it difficult to give an answer to the question of whether the substances formed in the organism of molluscs in response to the injection of tissue proteins of Chordata are true antigens, and what is the degree of specificity of these substances. In order to answer these questions further experiments are necessary, using, in particular, methods such as the adsorption of antibodies, and also immunochemical investigations. We are continuing to study this problem.

SUMMARY

The authors discuss the possibility of immune antibody production by marine invertebrates. Molluscs were immunized by extracts of Chordata (Styela rustica, Molgula retortiformis, Boltenia echinata), animals of higher organization than the molluscs. It was shown with the aid of the ring precipitation test that the corresponding antibodies appear in the celomic fluid of experimental animals 7-12 days after the completion of immunization. No appearance of antibodies was noted when more highly organized animals were immunized with the tissue of animals with a lower level of organization.

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MORPHOLOGICAL CHANGES IN THE LYMPHATIC GLANDS AND SPLEEN OF GUINEA PIGS REPEATEDLY INOCULATED WITH Rickettsia burneti (THE MORPHOLOGY OF IMMUNITY)

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From their studies of the conditions and laws of inhibition of immunological activity, P. F. Zdrodovskii [1, 2] and workers in his laboratory [4] have concluded that as a result of immunizational overexcitation with an antigenic stimulus of excessive strength, a state of reactive depression develops, which they associate with a varying degree of exhaustion of the reactivity of the recipient. Manifestations of immunological depression are also regularly reproduced, according to their findings, in all cases when one immunizing stimulus is significantly or highly predominant over others, especially when reacting successively with each other. The morphological reactions of these phenomena have not been studied.

The present investigation is an attempt to determine the character of the morphological changes in the lymphatic glands and spleen of guinea pigs during superinfection.

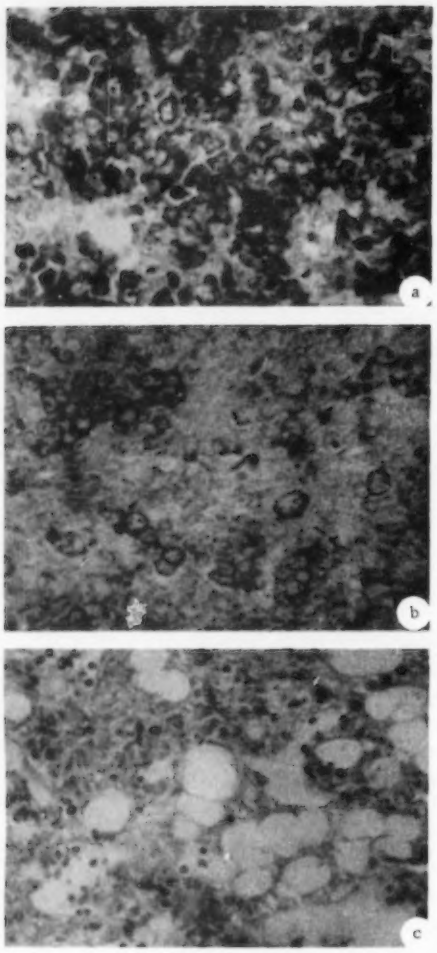
METHOD

Experiments were carried out on 40 guinea pigs weighing 250-300 g. The animals were preliminarily inoculated subcutaneously in the left inguinal region with a strain of Rickettsia burneti of low virulence (strain BD-2520, Gudima O. S., 1956) in a dose of 10,000 conventional guinea pig units. Thirty days later the animals were again inoculated subcutaneously in the right inguinal region with a highly virulent strain of R. burneti (Italo-Greek strain) in a dose of 100,000 conventional guinea pig units. Guinea pigs were sacrificed (two at a time) for morphological investigation on the 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 16th, 20th, 25th, 30th, 40th, 50th, and 60th days after the second inoculation. As a preliminary measure 2 ml of blood was taken from the heart of all the animals for serological investigation. The regional and common mesenteric lymphatic glands and spleen were examined. These organs were fixed in Zenker's formol and embedded in paraffin wax–celloidin. Sections were cut to a thickness of 5 μ and stained with azure II–eosin.

RESULTS

Macroscopically, at the site of the second injection, from the eighth to the 14th days isolated petechiae were seen in the connective tissue layer of the skin and the adjacent subcutaneous connective tissue. The regional lymphatic glands were enlarged, especially during the first six days after the injection; after 14 days they could no longer be seen to be enlarged. The spleen was firm, and slightly enlarged on the first few days; after 10-14 days it was normal in size.

Microscopically, in the lymphatic glands, especially the regional glands, large numbers of transitional cells [3] and plasmoblasts could be seen on the second day after inoculation (see figure, b). These cells were particularly numerous in the cortical zone of the lymphatic glands and in the lymphatic follicles. The cells of the reticular tissue were swollen, and many of them were in a state of mitosis. Another characteristic feature was the



Morphological changes in the organs of the guinea pig after two inoculations with Rickettsia burneti. a) spleen on the second day (large numbers of plasmoblasts and immature plasma cells are seen); b) regional lymphatic gland on the second day (transitional cells and plasmoblasts are seen); c) regional lymphatic gland on the 16th day after the second inoculation (atrophy of the medullary cords of the medullary zone). Photomicrograph. Ocular 20×, objective 20×. Stained with azure II–eosin.

presence of numerous mitotic divisions of immature plasma cells. The sinuses of the cortical zone of the lymphatic glands were firmly packed with large lymphocytes and plasmoblasts. Hardly any small lymphocytes were encountered here.

The same picture was observed on the fourth day also, although the mitotic activity of the reticulum cells was appreciably reduced.

On the sixth day, at the sites of accumulation of transitional cells and plasmoblasts, large numbers of adult plasma cells were seen, and these became predominant. The cortical zone of the lymphatic gland was appreciably thinned, the sinuses of the medullary zone were dilated and empty, and the medullary cords were tightly packed with plasma cells.

After the 8th-10th day the number of transitional cells and plasmoblasts fell sharply. The predominant cells became adult plasma cells and their degenerative forms. The number of reticulum cells also fell considerably and hardly any of these cells showed mitotic division.

After the 12th-14th day the comparatively rapid disappearance of all forms of plasma cells from the lymphatic glands was seen, especially in the regional glands. This was observed most clearly in the medullary cords of the medullary zone; on the 16th day all the plasma cells here had disappeared almost completely. As a result the medullary cords were largely atrophic, and appeared as fibrous structures with a comparatively small number of weakly stained reticulum cells (see figure, c). These morphological changes in the lymphatic glands were observed in all successive times of investigation until the 50th day. On the 60th day the medullary cords of the medullary zone of the lymphatic glands were almost normal, i.e., contained many reticulum and plasma cells, but there were comparatively few lymphocytes.

Roughly the same changes were observed in the spleen. On the second day after the second inoculation the entire red pulp of the spleen was diffusely packed with transitional cells and plasmoblasts (see figure, a), among which many mitoses were visible. On the sixth day, however, at the site of the previous accumulations of these cells mainly adult plasma cells were present, arranged more or less in nodes around the trabeculae and lymphoid follicles and under the capsule of the spleen.

On the 12th day comparatively few plasma cells were observed in the red pulp, but the nodal nature of their distribution was even more pronounced. Subsequently, until the 60th day, no changes whatever could be seen. By its morphological structure, the spleen at this time showed no particular difference from the spleen of control, uninoculated animals.

At the moment of the second inoculation, the mean titers of complement-fixing antibodies in the serum of the experimental animals was 1:60. After the second inoculation an increase in the antibody titers took place on the sixth day (1:160), reaching its maximum on the 12th day (1:320). A gradual fall in the antibody titers then gradually supervened; on the 40th and 50th days their level was 1:30, and on the 60th day it had risen to 1:120.

As the foregoing account shows, the subcutaneous injection of two large doses of R. burneti caused an intensive proliferative reaction of the cells of the reticular tissue of the lymphatic glands and spleen, with the almost complete transformation of these cells into plasma cells. The subsequent pattern of the morphological changes consisted basically of the rapid maturation and disappearance of the plasma cells thus formed. This process passed through a stage of degeneration of the plasma cells. The cessation of the formation of the transitional cells and plasmoblasts, on the one hand, and the accelerated, large-scale maturation of these cells into plasma cells followed by a relatively rapid disappearance of the latter, on the other hand, created the picture of atrophy of the reticular tissue, which was particularly prominent in the lymphatic glands.

As a result of this, the medullary cords of the medullary zone of the lymphatic glands, for example, were almost completely denuded of cells and consisted largely of fibrous structures with a few, extremely weakly stained cells (16th day after inoculation). It was only on the 60th day after inoculation that the medullary cords of the lymphatic glands of the experimental animals showed an arrangement of reticulum cells, capable of undergoing transformation into plasma cells, that was more or less normal in appearance and density. These latter were relatively numerous here.

The curve of titers of complement-fixing antibodies in this experiment followed the same line as the intensity of the plasma-cell reaction, lagging behind it by 6-7 days.

In the author's opinion, the most interesting face in this investigation is the temporary atrophy of the reticular tissue, particularly prominent in the lymphatic glands. This atrophy coincides in time with the lowest level of the titers of complement-fixing antibodies. Manifestations of immunological depression thus take place after repeated antigenic stimulation of considerable strength. The repeated injection of a large dose of R. burneti of high virulence caused an intensive but transient proliferation of reticulum cells followed by their subsequent rapid transformation into plasma cells. The impression is thereby created that the stocks of reticulum cells capable of such a transformation were apparently expended. The previous injection of a strain of Rickettsias of low virulence evidently prepared the reticulum cells for such a transformation. The replacement of the expended reticulum cells was retarded as a result of the second massive antigenic stimulus. The most likely hypothesis is thus that the manifestations of immunological depression are evidently associated with delay in the reproduction of reticulum cells capable of undergoing transformation into antibody-forming cells, i.e., plasma cells.

SUMMARY

Superinfection, induced in guinea pigs with a large dose of R. burneti, was accompanied by a brief rise of the complement fixing antibody titer (on the 6th–12th day) and a subsequent drop (on the 12th–50th day) and a new rise (on the 60th day). Morphological changes in the lymph nodes and spleen were characterized by a turbulent but brief proliferation of reticulum cells for a period of 2–4 days with subsequent rapid transformation of most of them into plasma cells. In this way it seemed as if the stocks of reticulum cells, capable of such transformation, were expended. The quick and almost simultaneous maturation of plasma cells leads to their rapid disappearance by degeneration. Temporary atrophy of reticular tissue especially noticeable in the medullary zone of the lymph nodes, resulted. The author considers that this temporary atrophy is connected with the state of immunological depression. The latter evidently results from the inhibition of the processes involved in the formation of reticulum cells, capable of transformation into antibody-producing cells, i.e., into plasma cells.

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RESPONSE OF RABBITS WITH IMPLANTED BROWN-PEARCE TUMOR TO VACCINATION WITH LIVE TULAREMIA VACCINE

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In a previous communication [1] we showed that the formation of immune complement-fixing antibodies in rabbits with an implanted Brown-Pearce tumor was depressed by comparison with the situation in healthy animals. The state of immunogenesis is only one of the indices of the defensive powers of the animal body and does not fully reflect the reactivity of an animal in which a malignant tumor is developing. We know from the literature [3] that post-vaccinal immunity in tularemia is due to the reorganization of the body as a whole and does not depend purely on the reaction of the reticulo-endothelial system or of humoral factors; an important place in the pathogenesis of tularemia is occupied by increased sensitivity to the causative agent of this disease, i.e., by allergy.

In this research we studied the reorganization of the reactive powers of rabbits inoculated with Brown-Pearce tumor and compared them with the response of healthy rabbits, resulting from post-vaccinal immunity against tularemia. The immunity of the rabbits vaccinated with live vaccine was determined by allergic and serological reactions.

METHOD

Experiments were carried out on male chinchilla rabbits of identical weight. Ten rabbits in the first group were inoculated intratesticularly with 0.5 ml each of a 25% suspension of Brown-Pearce tumor cells. A second group, also consisting of 10 rabbits, acted as controls. Six days later, when the development of a tumor could be determined clinically in the experimental group, the rabbits of both groups were vaccinated with a dried live tularemia vaccine, produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology (Series No. 492). Vaccination was performed intravenously, once only, each animal receiving an injection of 2×10^9 bacterial cells. The trend of the state of post-vaccinal immunity was studied in accordance with two indices—the allergic test for tularemia and the formation of agglutinins.

Blood was taken for performance of the agglutination test simultaneously with the allergic test to tularin on the 10th, 13th, 18th, 24th, 30th, and 36th day after the animals of the experimental group were inoculated with the tumor. The allergic test was performed as follows: The hair on the rabbit's flank was shaved, the site of the injection was painted with alcohol, and then tularin was injected intradermally through a fine needle in a dose of 0.1 ml (100 million bacterial cells). The reaction was read after 24 and 48 hours and evaluated by plus signs: a reaction of slight hyperemia and edema less than 1 cm in diameter—+, an area of hyperemia and edema 1 cm in diameter—++, and larger than 1 cm in diameter—+++. The second injection of tularin was given on the opposite side of the trunk, and the subsequent injections were made on alternate sides.

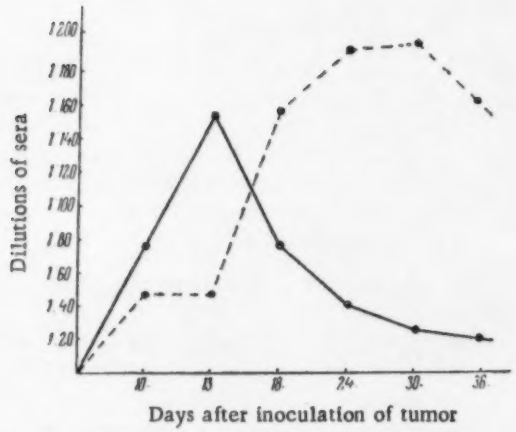

Fig. 1. Changes in agglutinin formation in the sera of rabbits with an implanted tumor and of healthy rabbits after vaccination with tularemia vaccine. Conventional signs: —— experimental animals; – – – – controls.

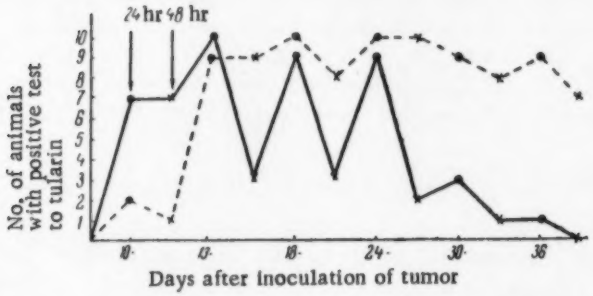

Fig. 2. Changes in the allergic reactions in rabbits with an implanted tumor and in healthy rabbits after immunization with live tularemia vaccine. Conventional signs: —— experimental animals; – – – – controls; x, ·— reactions read 48 and 24 hours respectively after injection of tularin.

The agglutination reaction was carried out with tularemia diagnosticum. The sera of the experimental and control groups were tested at the same time. Into each tube was poured 0.5 ml of rabbit serum, diluted by the volume method from 1:10 to 1:1280. Next, to all the tubes was added 0.5 ml of a 10^9 suspension of diagnosticum. The control tubes contained 0.5 ml of antigen and 0.5 ml of physiological saline. The rack with the tubes was shaken and incubated at 37° for 2 hr. The results of the reaction were then read and the tubes were kept at room temperature for 24 hr. The results of the reaction were then read again.

An agglutination reaction in which the liquid was completely transparent and no conglomerate developed on shaking was denoted by ++++. In the presence of two or three large clumps of agglutinate and slight opalescence of the liquid above the precipitate, the reaction was counted as +++. When the liquid above the precipitate was perceptibly cloudy and the precipitate was composed of loose floccules, the reaction was denoted by ++. A reaction of + was one in which turbidity was observed above the precipitate and characteristic floccules spread all over the liquid after shaking. In the discussion of the results of the agglutination reaction and the allergic test to tularin, we took reactions of ++ and above to be positive.

We took note of the time of death of the rabbits from metastases during the experiments and of the postmortem findings.

RESULTS

In Fig. 1 we show the results of investigation of the agglutinin titer in the sera of the experimental and control groups of rabbits after immunization of these animals with live tularemia vaccine. Along the axis of abscissas is denoted the day after inoculation of the Brown-Pearce tumor on which blood was taken for obtaining serum and the agglutination reaction was performed; along the axis of ordinates are plotted the titers of the sera. The curve in the form of a continuous line shows the mean titers of the sera obtained in the agglutination reaction in the experimental group (animals with an implanted Brown-Pearce tumor). The equivalent data for the control animals (healthy rabbits) are shown by the broken line.

It is clear from Fig. 1 that the titers of immune agglutinins in the experimental group reached their maximum value of 1:150 on the 13th day after inoculation of the tumor. With the further development of the tumor, the antibody titers began to fall sharply, whereas in the control rabbits they gradually increased. On the 30th day the titers of the sera of the experimental rabbits fell to 1:24, whereas the titers of the control sera amounted to 1:200.

It is interesting that in the first period of development of the tumor, the reactivity of the experimental animals was higher than that of the controls, for immunogenesis took place more intensively in the former; this is shown by the higher peak of the antibodies in their sera than in the controls (10th-13th day after inoculation of the tumor). These indices are in agreement with the findings obtained from a study of the allergic response of the experimental and control animals.

In Fig. 2 we show the results of experiments with the diagnostic test to tularin. Along the axis of abscissas are denoted the days after inoculation of the Brown-Pearce tumor on which the intradermal tularin test was carried out; along the axis of ordinates is plotted the number of rabbits in which a positive reaction to the intradermal injection of tularin was observed. The mean results of the allergic test to tularin in the experimental group are shown by a continuous line, and those in the control group by a broken line. A black circle on the curves indicates an allergic test carried out 24 hr after intradermal injection of tularin, and a cross—a test 48 hr after the injection.

Examination of the results given in Fig. 2 shows the following feature, which confirms the experiments on the study of immunogenesis.

On the 10th day after inoculation of the tumor the allergic test to tularin was positive in seven experimental rabbits, whereas it was positive in only two controls. Subsequently, until the 30th day, positive tests to tularin when read 24 hr after injection were observed in roughly the same number of animals in the experimental and control groups. Starting on the 13th day, however, sharp differences in the extinction of the allergic reaction were found.

After 48 hr, in the rabbits with an implanted tumor, the tularin test either became negative or was assessed at +, whereas in the control group it maintained its intensity even for 72 hr in the majority of animals. This index was so regular that we later used it to establish the prognosis regarding death of the rabbits with an implanted tumor, and it was always found to be right.

When most of the animals' organs were affected by metastases (30th-36th day), the tularin test in fact became negative when read after 24 hr in nearly all the rabbits with an implanted tumor.

These findings are in agreement with the observations described by other authors [2, 4]; they demonstrate that the tumor itself is a protein sensitizer, modifying the reactivity of the animal. In the first period of development of the tumor the reactivity of the animals is therefore increased, which accounts for the stimulation of immunogenesis and the allergic reactions on the 10th-13th day after inoculation. Subsequently, as metastases become widespread, allergy is depressed and may even proceed as far as its irreversible form—anergy.

In our experiments vaccination with live tularemia organisms induced additional sensitization. In consequence of this, the rabbits with an implanted tumor became hypersensitized, which was shown by a considerable depression of their reactivity. At the end of their life a state of total anergy developed and they lost the power both of immunogenesis and of manifestation of allergic reactions.

SUMMARY

The reactivity of rabbits with an implanted Brown-Pearce tumor was studied following their vaccination with live tularemia vaccine. Formation of immune agglutinins and allergic reactions were investigated both in healthy rabbits and in animals with an implanted tumor. The study of these reactions demonstrated an intensified reactivity of the experimental animals during the first stage of tumor development; its marked depression up to the time marking the development of anergy was noted during metastasis.

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* Original Russian pagination. See C. B. translation.

IMMUNIZATION OF MICE WITH A TUMOR GROWN ON THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO

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Many workers have shown that the most stable immunity to a tumor can be attained only after a certain degree of growth of the tumor, followed by its absorption [1, 2, 3]. A great variety of methods has been used in order to produce experimental absorption of the tumor: intradermal injections, interference with the blood supply of the tumor, the injection of chemicals into it, and so on. Nevertheless, these methods of immunization against tumors have not always been successful, for instead of the desired result of absorption of the tumor, its progressive growth has been observed [1, 6, 7].

In the present research, in order to produce immunity to a tumor, we decided to use an Ehrlich's carcinoma grown on the chorioallantoic membrane of the chick embryo. No similar work has been reported in the literature.

When we studied the biological properties of a tumor cultivated in the chick embryo, we observed [5] that in the coarse of passage the tumor acquires the property of undergoing absorption when reinoculated in the original animals in 80% of cases. This property was found at the fifth passage of the tumor in the embryo. We therefore thought that, after passage in this way, such tumors could be used for immunization.

METHOD AND RESULTS

For immunization we used male white rats weighing 24-25 g. Altogether four groups of mice were immunized; two were controls. Each group consisted of 112 animals. The first group included mice in which a tumor, inoculated after the fifth passage in the chick embryo, was undergoing absorption. The animals of the second group were immunized with tumor material after the fifth passage in the chick embryo and treated with 0.2% formalin solution. The third group consisted of mice immunized with Ehrlich's carcinoma, not cultivated in the chick embryo, treated with the same formalin solution. The animals of the fourth group were immunized with tissue from the chorioallantoic membrane of a 14-day old chick embryo.

Immunization was carried out by the subcutaneous injection of a 10% tumor suspension in the dorsal region. The dose injected was estimated as protein, which was determined by Conway's method. Each animal received 3-4 mg of protein in 0.2 ml of 10% tumor suspension.

From 10 to 15 days after immunization (in the first group—after absorption of the tumor) all the mice received a second inoculation of the ascitic form of the tumor intraperitoneally in a minimum inoculating dose. This dose was preliminarily titrated in mice; in our experiments the dose was 0.2 ml of ascites fluid diluted 1:1000. For the reinoculation we used as a rule a slightly larger dose of tumor in order to obtain a 100% take.

After reinoculation, we noted the time taken for an obvious ascitic tumor to develop, the character of its subsequent growth and the time of death of the animals.

Immunization of Mice with Tumor After Passage in the Chick Embryo

Group of experiments	Material used for immunization	Time of appearance of visible ascites (in days)	Time (in days) of death of mice	
			after reinoculation	after appearance of ascites
First	Inoculation of tumor after 5th passage with subsequent absorption . . .	18–21	31–36	15
Second	Tumor after 5th passage, treated with 0.2% formalin	8–11	18–23	11
Third	Tumor, not subjected to passage, treated with 0.2% formalin	8–11	17–23	11
Fourth	Tissue of chorioallantoic membrane of 14-day old chick embryo	10–11	18–24	11
Fifth	Material control, inoculation of minimum dose of ascites fluid . . .	8–11	18–24	12
Sixth	Technical control, inoculation of usual doses of ascites fluid	5	14–17	10

Note. Mean times of death of the mice are shown in the table.

The rate of growth of the ascitic tumor was also judged by the change in the circumference of the abdomen of the mice and by their increase in weight. By this means the changes in tumor growth could be detected sooner. In order to determine the significance of the results obtained, a statistical analysis was made of the survival rate of the mice of the experimental and control groups by the Fisher-Student method. Altogether we carried out 4 series of experiments on 672 mice.

The method which we used enabled us not only to study the immunizing properties of the Ehrlich's tumor after the fifth passage in the chick embryo (first group of experiments), but also to compare the immunizing properties of the "living" and the formalinized tumor tissue (second and third groups of experiments). We could, moreover, examine the immunizing properties of tumor tissues, both subjected to passage (as in the second group of experiments) and not (third group of experiments), and treated with formalin.

Immunization with tissue of the chorioallantoic membrane was undertaken in order to exclude its immunizing influence after absorption of the tumor after the fifth passage. The possibility of the production of relative immunity to a tumor after immunization with embryonic tissues is well known from the literature [4].

When giving the injection to detect the presence of immunity, we considered it essential to use minimum inoculating doses. The use of large doses may lead to the suppression of an immunity the degree of which is unknown.

The results of the experiments are shown in the table. In it are recorded the times of appearance of visible ascites fluid, of death of the mice after the inoculation to detect immunity, and of death of the mice after the appearance of visible ascites fluid.

In the first experimental group ascites fluid was found on the 18th-21st day after inoculation, on the average 10 days later than in the mice of the control group. Somewhat different results were obtained during the study of the times of appearance of ascites fluid in the mice of the other experimental groups.

The times of appearance of ascites fluid in the animals of all these groups were the same, and they coincided with the moment of formation of visible ascites fluid in the control mice. For example, in mice immunized with formalin-treated tissue from a tumor not subjected to passage, ascites fluid appeared on the 8th-11th day, i.e., at the same time as in the control animals.

Thus in mice immunized with the tissues of a tumor after passage, the appearance of ascites fluid was later than in the control animals. The fact that the latent period of development of the tumor in these mice was prolonged cannot be explained by weakening of the virulence of the strain, for growth of the ascitic tumor was observed as usual in the control animals: appearance of the tumor on the fifth day and death of the mice on the 14th-17th day after inoculation of the tumor.

Consequently, the prolongation of the latent period of development of the ascitic form of the tumor is the result of immunization of the mice with tissues of the tumor after passage.

Similar results were also obtained from the study of the time of death of the animals. The mice of the first experimental group died on the 31st-36th day after reinoculation of the tumor, i.e., 12 days later than the controls. The control animals died on the 18th-24th day after inoculation. At these times death of the animals in the remaining experimental groups was also observed.

Thus the study of the time of death of the mice also revealed a lengthening of their survival period in the principal experimental group, not only by comparison with the control animals, but also with the animals of the other experimental groups. After statistical analysis of the figures showing the time of death of the mice in all the experimental groups and the controls, only the figures of the survival of the mice in the first experimental group were significant ($P = 0.00001$). By studying the time of death of the mice after the appearance of ascites fluid it was impossible to find any significant difference between the experimental and control figures. The mice of the first experimental group died on the 15th day after the development of ascites fluid. The control animals and the mice of the remaining experimental groups died slightly earlier—on the 11th-12th day.

The study of the immunizing properties of an Ehrlich's tumor after the fifth passage in the chick embryo thus showed that a relative vaccinating effect could be obtained, as shown by the prolongation of the survival of the experimental animals and by the slower rate of growth of a reinoculated tumor. This is confirmed by an increase in the latent period of development of ascites in the experimental mice by comparison with the controls. The experiments also showed that positive results may only be obtained from immunization in the case of adsorption of the implanted tumor.

SUMMARY

The author studied the immunizing properties of Ehrlich's carcinoma after the 5th passage in the chorioallantoic membrane of a chick embryo. As a result of such immunization the life-span of experimental animals may be prolonged and the growth rate of a reinoculated tumor changed. Positive results of this vaccination were noted only after the resolution of the tumor used for immunization.

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A COMPARATIVE CYTOLOGICAL ANALYSIS OF THE TISSUE CULTURE CELLS UNDER NORMAL CONDITIONS AND ACTED UPON BY THE POLIOMYELITIS VIRUS

REPORT II. CYTOLOGICAL CHANGES IN THE GROWING CELLS ACTED UPON BY THE POLIOMYELITIS VIRUS

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In our first report [3] we presented data on the dynamics of normal growth changes for 4 cell lines cultured outside the organism, kidney cells from Macacus rhesus (2nd generation), a strain of heart cells from Macacus cynomolgous, and strains of the malignant cells HeLa and HEp-2. In this report we present the results of studying externally cultivated cells acted upon by the poliomyelitis virus.

A considerable amount of work was devoted to studying the morphological changes caused in the tissue culture cells by the different viruses. A survey of the existing data may be found, in particular, in the abstract by Syverton [9]. However, these investigations were not carried out on a comparative basis, and the authors were much more interested in the phenomenology of the cytopathogenic effect than in its dependency on the properties and the state of the effected cells. This situation stimulated us to investigate the problem from its comparative-cytological aspect and to study the cultures at different stages of their life cycle.

METHOD

The cells were cultured on layers of mica (moscovite) in a fluid nutrient medium. The cultivation conditions and the histological technique employed have been described in our previous report [3]. The cultures, containing 50,000 cells per ml, were inoculated with the poliomyelitis virus (type I, Brunden strain), 100-50 TsPD. The virus was added to the suspension of cells prior to the beginning of cultivation, and also to the growing cultures at various stages of cultivation. Material for the morphological investigation was taken at 1, 4, 8, 12, and 24 hr after inoculation with the virus and then daily until the end of the cultivation. Fixation was carried out in Bouin's solution, and hematoxylin and eosin were used for the staining.

RESULTS

I. Addition of Virus to the Cell Suspension Prior to the Beginning of the Cultivation

Despite the action of the virus, the cells attached themselves to the mica and formed a significant layer on its surface. The changes observed in their morphology have been considered for each culture individually.

1. The Culture of Renal Cells of Macacus rhesus. One day after inoculation of part of the cells we observed significant changes, predominantly concerned with the structure of the nucleus: its membrane lost turgor, and in it there appeared compact accumulations of chromatin, lying principally under the membrane. The shape of the cells also changed: they became rounded or acquired branch-like formations, which gave them a certain similarity to neurons. The protoplasm was often eosinophilic, and inclusions could be seen in it, distributed either compactly or diffusely (Fig. 1a). With a greater degree of damage we encountered abnormal accumulations of chromatin in the cytoplasm, sometimes simulating the picture seen with mitosis. In the later stages, after 2 days, only groups of single, markedly altered, rounded cells, their "shadows," remained in the layer (Fig. 1b).

2. The Culture of a strain of Cells from the Heart of Macacus cynomolgous (SOTs). The effect of the virus appeared differently from that seen in the culture of kidney cells. After cultivation with the virus for a day changes were manifested mainly in the peculiar round form of the cell clumps, appearing like "beads" (Fig. 2a). At the same time, the first signs of specific degeneration, described above, appeared in the nuclei of several cells. On the second day the number of cells altered in this way had increased, and by the 3rd and 4th days we observed only single, markedly altered cells in the layer.

3. HeLa Strain of Cells. In the course of the first days of viral action the typical changes described above were not present; all that was noted was the appearance of a small number of compact and rounded cells, isolated from the layer (Fig. 2b). On the 3rd day of cultivation there appeared a significant number of pathologically altered cells. The normal cells remained only in the form of small islets.

4. HEp-2 Strain of Cells. A similar pattern of changes took place in the cells of the culture HEp-2 as well. A small number of typically altered cells appeared only on the 2nd day. On the third day approximately 50% of the cells were pathologically altered. By the 4th day only a small number of markedly changed cells were left in the layer.

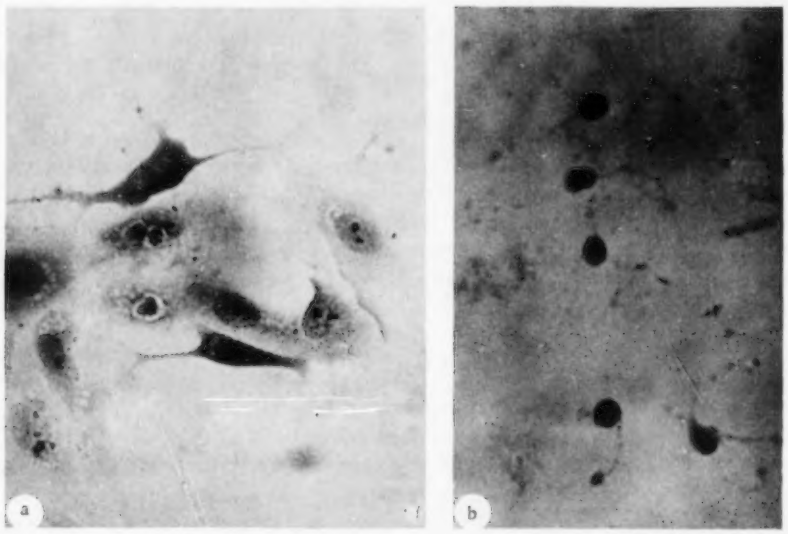

Fig. 1. The effect of Poliomyelitis virus (Type I, Brunden strain) on the cells of a subculture from the kidney. a) After a 24 hr exposure to its action; b) after a 96 hr exposure. Fixation was carried out in Bouin's solution; staining was performed with hematoxylin and eosin. Photomicrograph. Magnification 350×.

II. The Effect of the Virus on Growing Cells

In this series of experiments we studied the effect of the virus on cells that had been growing for varying lengths of time (1–8 days) in the cultures. We noted significant morphological changes in the cells, qualitatively

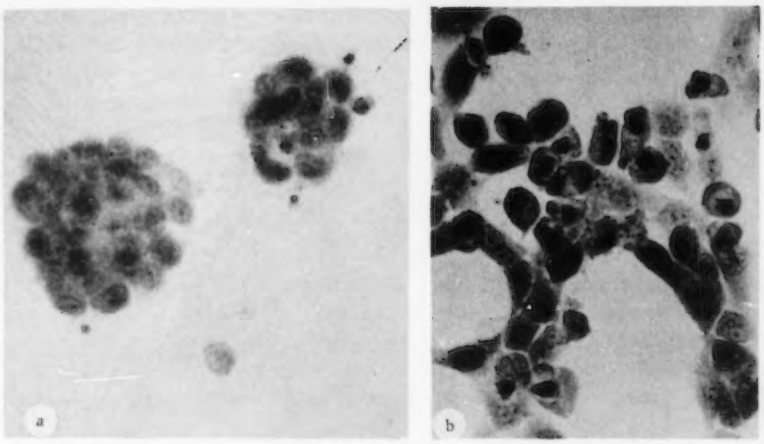

Fig. 2. The effect of Poliomyelitis virus (Type I, Brunden strain). a) On cells of the SOTs culture after a 24 hr exposure. Fixation was carried out in Bouin's solution; staining was performed with hematoxylin and eosin. Photomicrograph. Magnification 350×.

TABLE 1

The Dynamics of Changes in the Number of Monkey Renal Cells Damaged by the Poliomyelitis Virus as Related to the Duration of Exposure and the Age of the Culture (per 1,000 Uninvolved Cells)

Age of the culture (in days)	Duration of exposure to the virus (in days)			
	1	2	3	4
1	244	207	Almost all involved	Almost all involved
2	232	210	444	342
3	165	273	743	Almost all involved
4	125	123	124	151
5	183	—	200	—

TABLE 2

The Dynamics of Changes in the Number of SOTs Cells Damaged by the Poliomyelitis Virus as Related to the Duration of Exposure and the Age of the Culture

Age of the culture (in days)	Duration of exposure to the virus (in days)		
	1	2	3
1	206	Almost all cells involved	No. cells left on the layer
2	64	The same	The same
3	56	175	—

similar to those described above but differing from them, somewhat, principally in their dynamics.

1. The Culture of Renal Cells of Macacus rhesus. The first statistically reliable changes due to the virus were observed only after 12 hr following the beginning of the exposure. However, isolated typical cell changes were encountered in the culture after exposure to the virus for 8 hr. One day after the initiation of the viral action we noted a considerable number of typically changed cells. The number of these cells gradually increased over the next few days of the exposure. By the 5th day we found only a small number of cell "shadows." Beginning with the 6th day there was complete absence of the culture treated with the virus from the layer.

2. The Heart Strain of Cells (SOTs). As in the exposure of the cells in suspension, the changes were reflected primarily by alterations in the form of the cell agglomerates. Changes in the morphology of the separate cells could be noted only on the 2nd day. They decreased in size, became rounded, their cytoplasm became markedly vacuolated, the nuclei, more compact and occasionally containing compact accumulations of chromatin, and, in several instances, clumps of chromatin lay directly in the cytoplasm.

3. The HeLa Strain of Cells. Beginning with the 2nd day of exposure changes were noted in the character of cell distribution: there were no heavy layers, the cells were compact, and by the 2nd day the signs of typical degeneration appeared in isolated cells (Fig. 3). The number of altered cells gradually increased. Nevertheless, a small number of cells were observed on the layers even at the end of the experiment (7 days).

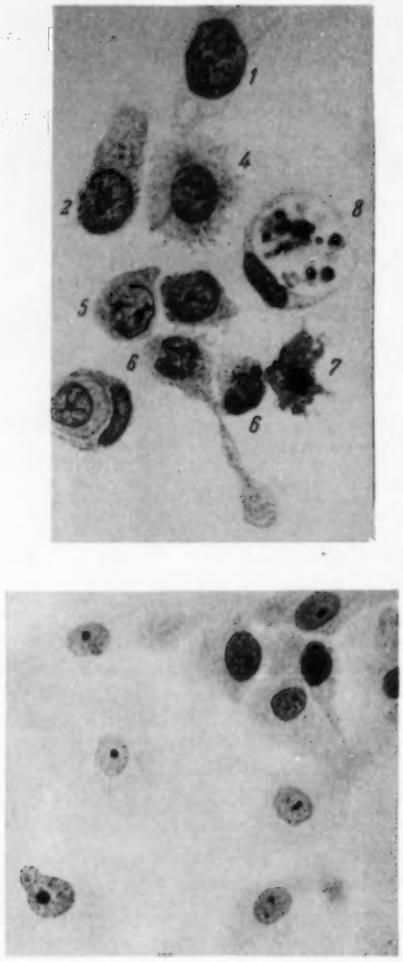

Fig. 3. Successive stages (1–8) in the development of degenerative changes of the nucleus and cytoplasm in HeLa cells (above). Drawing apparatus. Magnification 315×.

TABLE 3

Stimulation of Mitotic Activity in Cells Cultivated Outside the Organism Under the Influence of the Poliomyelitis Virus (Type I, Brunden strain, 100-50 TsPD). Number of Mitoses per 1000 Culture Cells

Age of the culture (in days)	SOTs	HeLa	HEp-2
1	27	41	22
2	85	78	87
3	42	31	21

4. The HEp-2 Strain of Cells. As in the case of the HeLa cells, compact rounded cell accumulations already appeared by the 1st day. However, they did not show the typical changes in the nucleus or protoplasm. On the 2nd day the typical signs of degeneration appeared in isolated cells.

In all the cultures investigated the damaged cells arranged themselves in compact groups between the morphologically normal cells.

So as to elucidate the dependency of the morphologically changed cells, exposed to the virus, on the age of the culture, we added the virus to the culture after varying durations of growth and fixed material each day, beginning with the 1st day of exposure and continuing until the occurrence of senile degeneration in the control cultures. Hoping to study the relationship of the involved cells to the age of the culture objectively, we counted the number of damaged cells in the preparation per 1,000 uninvolved cells. The data obtained for the cells from the renal cultures is presented in Table 1.

We observed similar dynamics for the changes in the culture of SOTs exposed to the virus (Table 2).

Analysis of the material in Tables 1 and 2 shows that sensitivity to the action of the virus, manifested by the morphological changes, was highest in the comparatively young cultures, under 3 days old. High sensitivity was indicated by two signs: 1) the number of damaged cells in the young cultures, after any given time interval, was higher than in the older cultures; 2) in the young cultures the effect of the involvement developed more rapidly and by the 3rd day had already led to massive degeneration of the cells. In contrast, cell damage in the 5-day old cultures was manifested in a comparatively small number of cells (12–18%) during the first few days (see Fig. 3), and that picture lasted, without particular changes, up to 4 days of cultivation with the virus. The reason for the decreased vulnerability of the cells in the several-day-old cultures is still not clear, although it may be postulated that the intense metabolic processes peculiar to cells in the first stages of cultivation potentiates the elevation in their sensitivity to the viral action.

III. Changes in the Mitotic Behavior of the Cultures Subsequent to the Action of the Poliomyelitis Virus

The action of the virus on a suspension of the cells being cultivated (especially the cells for the cultures of SOTs, HeLa, and HEp-2, distinguished by high numbers of mitoses) led, by the 2nd day, to a marked elevation in mitotic activity (Table 3). It may be postulated that this increase in mitotic activity is a reaction connected with

either a protective stimulation of the cells' metabolism when they are exposed to the viral action, or with the direct injurious action of the latter, which leads to local necrotic processes, which, in turn, may also serve as sources of stimulation for the mitotic activity [10]. It should be pointed out that, according to certain data, when cultures of monkey kidney cells are acted upon by the poliomyelitis virus, it results in a temporary increase in glycolysis [8], and an elevation in the acid phosphatase activity [7]. There are indications in the literature that the action of pathogenic and reactive factors causes a temporary stimulation of mitotic activity. Thus, it has been shown that injecting mice with tetanus toxin causes an elevation of the mitotic activity in the corneal epithelium after 36 hr [4]. The injection of anti-plague vaccine leads to an increase in the mitotic activity of the cells in the spleen [2]. The stimulation of mitotic activity in the culture cells was of a short-term nature; even by the 3rd day we noted its intensity subsiding, caused, apparently, by the fact that at that time the growth of the culture almost completely ceases.

An elevation in the mitotic activity by the second day of exposure to the virus was also noted in the cultures to which the virus was added when they had already begun to grow.

The activity of the poliomyelitis virus on a suspension of the cultured cells did not impede their attaching onto the layer or their first stages of growth, but, after several days, led to their death. Morphologically the action of the virus manifested itself by typical, successively developing, changes in the nucleus and cytoplasm, described in a number of works [6], particularly in the recent investigation by A. I. Drobyshevskaya and V. P. Mikhailov [1]. On the basis of morphological data, the authors came to a conclusion regarding the resultant changes in the virus-infiltrated cells which coincides with our hypothesis, the latter based on our observing the stimulation of mitotic activity.

Besides noting changes in the individual cells of the transplanted strains SOTs, HeLa, and HEp-2, we also observed changes in the character of their distribution. In place of uniform layers there appeared separate groups, containing several "tens" of cells. This, obviously, was a result of weakening the intercellular bonds. The observed disturbances, occurring as a result of the poliomyelitis virus, were morphologically different in the cells of the different cultures. In the cells from the culture of monkey kidney the changes had already begun within the first days of exposure, immediately effecting a considerable portion of the cells and rapidly increasing. By the third day there were almost no cells left on the layer. In the SOTs culture the changes did not appear in a significant number of cells until the second day of exposure, but the rate of subsequent development of the cell involvement was about the same for the two cultures. Cells from the strains that originated from malignant tumors reacted to the action of the poliomyelitis virus in a morphologically similar manner, albeit somewhat slower and less pronounced than the specimens from the suspension of normal cells. A sufficient number of typically altered cells appeared in these cultures only by the third day; complete cessation of growth in these cultures occurred approximately a day later than in the kidney and SOTs cultures.

It must be noted that the capacity of cultures to manifest morphological impairments under the influence of a virus does not always directly correspond to their sensitivity to the action of the virus as determined by virological methods. There are indications in the literature [5] that there is no coordination between disturbances in the structure of HeLa cells acted upon by the poliomyelitis virus and the accumulation of this virus in the culture. Derivatives of fluorine, for example, impede the multiplication of the virus, yet do not inhibit the appearance of the cytopathogenic effect. The authors came to the conclusion that the processes leading to the elevation of the viral concentration in the culture and the processes leading to the pathological changes in the cells were, to a significant degree, independent of one another.

The sensitivity of the growing cells to the virus was lower than that of the cells in the suspension. This was demonstrated by the preservation of a certain number of undamaged cells within the layer, even after extended intervals of cultivation. The high sensitivity to the viral action shown by the cells in the early stages of development, as compared with those already growing, is apparently related to the more intense metabolism which is characteristic for cells in their first stages of growth outside the organism.

SUMMARY

The authors studied the effect of polio virus upon the morphology of the cells obtained from subculture of the renal tissue (Macacus rhesus), heart (Macacus cynomolgus) and HeLa and HEp-2 malignant cells. The changes become noticeable in individual cells after an 8–12-hour action and are marked in 24 hr. These changes are

manifested in the chromatin redistribution, the loss of nuclear membrane turgor, the change of the cellular shape and cytoplasmic eosinophilia. The most sensitive to the action of the virus are the cultures during the initial stages of growth, and the youngest and the most active cells in a given culture. The following changes occur in the cultures of transplantable strains acted upon by the virus: at first decomplexation of the stratum is noted with the formation of roundish cellular accumulations, and only later the changes in the individual cells are seen. Temporary stimulation of mitotic activity is noted in the cells of the transplantable strains on the second day of the viral action

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COMPARISON OF INJURY TO SEXUALLY IMMATURE AND MATURE RATS BY HIGH DOSES OF X-RAYS

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Experimental proof has now been obtained that the reaction of the growing animal to ionizing radiation shows a number of fundamentally distinctive features. From a comparatively large number of facts in the literature, it is possible to speak of concrete differences in the reactions to irradiation characteristic of a particular age period [1-9]. The great majority of investigations deal with the study of the influence of doses producing acute radiation sickness [4, 7, 8, 9]. This is due to the necessity for problems in medical practice to be solved in their proper order of urgency. Accordingly, information on the action of small and very large doses of radiation on the growing animal is very scanty. Meanwhile it is obvious that the solution of the problem of age radiosensitivity may be achieved only by the investigation of the reaction of the animal to irradiation with a very wide range of doses. It is especially important to define the threshold doses, beyond the limits of which a qualitative change in the reaction of the animal to irradiation is observed.

In the present research an attempt is made to study the distinctive manifestations of the pathological processes arising in response to the action of large doses of x-rays, causing, on the one hand, the so-called intestinal death, and on the other hand, the development of the pathological condition termed by the majority of researchers "the acutest form of radiation sickness."

METHOD

We studied the action of large doses of x-rays on young rats aged 25-30 days (weight 50 g) and compared it with the action of the same doses on sexually mature rats aged 3-4 months (180-250 g).

Altogether we investigated 311 animals, of which 131 were sexually immature and 180 mature. As an additional control of the postmortem findings 21 unirradiated rats were used.

Irradiation was given by means of a RUM-3 apparatus, with voltage 180 kv and current 20 ma. No filters were used. The distance from the anode of the tube to the animal's spine of 24 cm was used rarely and only for the smallest rats. The experimental rats were placed in pairs at the moment of the experiment in specially made plywood boxes. We investigated the reaction to irradiation with x-rays in doses of 10,000, 15,000, 25,000, and 50,000 r. The dose rate varied from 249 to 475 r/min.

RESULTS

The results of the observations on the times of death of the animals are shown in Fig. 1, from which it may be seen, firstly, that the proportion of rats dying at a given period is determined by the age of the animals as well as by the size of the dose. If the course of the curves for each dose is compared for the sexually immature and

TABLE 1

Mean Duration of Survival of Rats Depending of Age and Dose of Irradiation (the figures in parentheses indicate the mean square deviation)

Dose of irradiation (in r)	Mean duration of survival of dying rats (in days)	
	sexually mature	sexually immature
10 000	3.5 (± 0.6)	3.0 (± 0.42)
15 000	3.5 (± 0.44)	2.8 (± 0.9)
25 000	1.6 (± 0.58)	1.3 (± 0.57)
50 000	1.0 (± 0.25)	1.0 (± 0.01)

TABLE 2

Figures Summarizing the Changes in Weight of the Rats at the Time of Death, Depending on their Age and the Dose of Irradiation

Dose of irradiation (in r)	Sexually mature rats		Sexually immature rats	
	wt of cadavers (as % of initial wt, taken as 100 %)		wt of cadavers (as % of initial wt, taken as 100 %)	
	limiting variations	mean value	limiting variations	mean value
10 000	73–94	83	58–86	72
15 000	69–89	79	64–95	79
25 000	80–97	88	87–90	88
50 000	91–97	94	76–96	86

mature rats separately, these differences can be detected, and as the dose increases they become obliterated. The differences were most marked at a dose of 10,000 r and least at 50,000 r. For example, after irradiation with a dose of 10,000 r, at the end of the first 24 hours 2% of the rats had died. At the end of the second day there were no further deaths, at the end of the third day the mortality rate reached 90%, and at the end of the fourth—100%. Consequently, the bulk of the young rats died in the course of the third day (90%). Meanwhile there were no deaths among the immature rats during the first day after irradiation with a dose of 10,000 r, at the end of the second day 3% had died, and at the end of the third day only 38% of the rats, i.e., the bulk of the rats died after the third day.

By analyzing in this way the course of the curves showing the mortality among the immature and mature rats after the action of various doses of x-rays, the greater vulnerability of the immature rats can be convincingly shown.

Although there is no doubt about the existence of differences on the basis of the results described, it must nevertheless be recognized that these findings are insufficient for the formation of a final conclusion on the importance of age as a factor capable of influencing the character of the radiation injury in animals when irradiated with such large doses of ionizing radiation. Other indices must also be taken into consideration. In Table 1 we show the figures giving the mean duration of survival of the animals depending on their age and the dose of irradiation.

It can be seen from Table 1 that the results in respect of the mean duration of survival of the rats after irradiation confirm the previous conclusion. In this case, moreover, the differences are of moderate degree.

The data summarizing the decrease in weight of the rats at the time of their death, shown in Table 2, are noteworthy.

The figures given in Table 2 show that as the dose increased the loss of weight of the young rats was less pronounced. This relationship also held good after irradiation of the sexually mature rats, although it was less obvious. These features are probably based on differences in the times of death of the animals, which also predetermine the degree of loss of weight: usually, the later the animal dies the more weight it loses. Comparison of the figures of loss of weight by the young and the mature rats shows that the young rats lost considerably more weight. It must be pointed out, however, that the difference was not significant, and in some cases (at doses of 15,000 and 25,000 r) it was absent altogether.

The clinical picture of the lesion and the postmortem findings were particularly interesting. In both the young and mature rats, after irradiation with a dose of 10,000 r, a severe form of radiation sickness developed, accompanied by extreme inhibition, adynamia, severe or total loss of appetite, and indifference to the surroundings. A leading symptom was severe diarrhea. The rats looked dirty, their hair was untidy, and their eyes inflamed and half-closed. At postmortem the typical picture of acute intestinal death was found. This took the form of severe hyperemia of all the internal organs, a considerable decrease in the size of the spleen and thymus, and gross dilatation of the stomach and of part of the intestine. It should be mentioned that the stomach, as a rule, was tightly distended with liquid food contents. In the lumen of the intestine a yellowish-pink liquid could

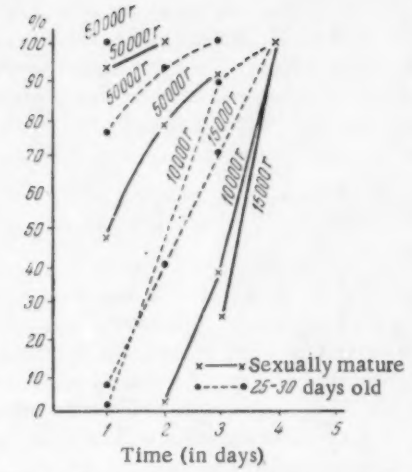

Fig. 1. Curves showing the percentage of rats dying at corresponding times, depending on their age and the dose of irradiation. Along the axis of ordinates—percentage of dying rats; along the axis of abscissas—time of death (in days).

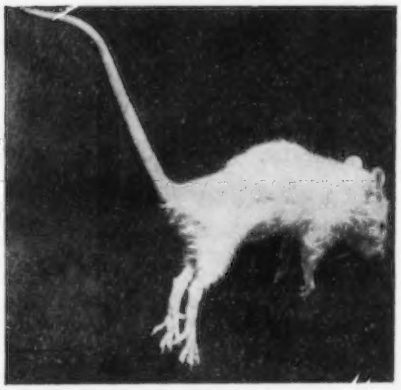

Fig. 2. Convulsions in a young rat 26 hr after irradiation with a dose of 25,000 r (experiment no. 566).

be seen through the thinned walls. In the lower divisions of the large intestine no formed fecal masses whatever were present.

Often in association with grossly hyperemic lung fields large effusions of blood without clearly defined borders could be seen. The brain was severely hyperemic but there were no hemorrhages.

After irradiation in a dose of 15,000 r, the sexually mature rats showed the same form of radiation sickness as after a dose of 10,000 r. In the young rats the course of the disease was much more severe. There was a high mortality among the animals on the second and, partly, on the first day, whereas among the sexually mature rats no deaths took place. Thus when a dose of 15,000 r was used, an obvious intensification of the pathological manifestations of the disease was found in the young rats. This dose apparently was the threshold dose, causing a lesion with signs of a qualitatively new form of reaction to irradiation, which was shown more clearly after irradiation with a dose of 25,000 r.

In the young rats, as a result of irradiation with a dose of 25,000 r, an extremely severe form of radiation sickness developed. Death took place mainly on the first and partly on the second day. Very rarely death occurred at the beginning of the third day. In the affected animals a new symptom—convulsions—appeared for the first time. The development of convulsions was preceded by the characteristic picture of severe radiation sickness. Usually immediately after irradiation the young rat became unable to orientate itself properly. Its movements were uncertain and incomprehensible. It usually moved around in a circle, inclined slightly on one side. In the course of time some weakness and folding in of the limbs could be observed. Shaking of the head and sometimes of the whole body was noted. The hair was untidy. In many animals there was involuntary micturition and defecation. Some animals gave out a squeak, probably on account of a sensation of pain.

In a number of animals severe excitation and restlessness were observed. Roughly ten hours after irradiation attacks of convulsions developed, and these became particularly severe in character at the end of the first and beginning of the second day. Signs of disturbance of the activity of the gastrointestinal tract increased considerably in intensity. The young rats were dissheveled and dirty from the severe diarrhea, and completely refused to take food. The fits gave way to periods of severe adynamia and inhibition. Some of the typical states observed in this period of development of radiation sickness are shown in Figs. 2 and 3.

Regarding the symptomatology of the radiation sickness in the mature rats, mention must be made firstly of the fact that a smaller proportion of them died on the first and second days. In some cases (in 8%) the rats even died on the fourth day. The picture of the condition bore the same character in the majority of animals. In some rats the typical convulsions described above did not develop. The character of the response reaction to irradiation with this particular dose thus was different in some rats. It must also be pointed out that convulsions developed on the average slightly later in the sexually mature rats. It is most probable that this explains the discovery at postmortem examination of some of the animals of typical signs of acute intestinal death.

The pathologoanatomical signs of the radiation injury consisted above all of vascular disorders of a very severe degree, expressed as severe dilatation of the vessels. Signs of stasis were present in the thymus, heart, intestine and adrenals and, in particular, in the lungs, where massive effusions of blood were found very often. The

Fig. 3. A shock-like state in a young rat 5½ hr after irradiation with a dose of 25,000 r (experiment no. 587).

spleen, which was considerably shrunken, was lighter in color in approximately one half of cases than usual. Because of the vascular disorders the liver appeared unevenly stained. The brain showed severe hyperemia and edema. No hemorrhages were present. Food contents were found, as a rule, in the stomach. Distension was present comparatively rarely, and was moderate in character.

The decrease in the size of the thymus, so typical of the response to lower doses, in these cases was moderate in character and sometimes was ill defined.

As a result of irradiation of the animals with a dose of 50,000 r, the picture of the condition was considerably aggravated. As a rule the animals died during the first 24 hr. Only in the group of the sexually mature rats was death of part of the rats (7%) after 24 hr observed. The earliest deaths among the young rats took place 5 hr after irradiation. Because of the great severity of the lesion, a clinical evaluation of the state of the animals from a comparative aspect was very difficult and often impossible, and accordingly we did not attempt to make one.

Most rats immediately after irradiation showed a characteristically rapid and heavy respiration, loss of orientation, circus movement, involuntary micturition and diarrhea. During these movements the animals suddenly fell on their side, got up again with difficulty and often crawled on their stomach, trailing their hind-limbs. This picture quickly changed into one of complete adynamia and severe inhibition. The animals lay on one side in uncomfortable positions and curled up or else, stretched themselves out in a weakened condition. The picture that developed could be described as that of a shock-like state. In these cases, often immediately after irradiation, the animals completely failed to react to the environment. Strong mechanical stimulation (pricking, pinching, pulling out of the hair) caused no response reaction. The animals did not, however, die at this period. In a number of cases immediately after irradiation excitation took place in the presence of the symptoms described previously. It was more severe in character than that described earlier. Convulsions and paralyses of the limbs appeared sooner, after 2-3 hours. They were very pronounced. Mechanical stimulation likewise caused a sharp response reaction. Even light contact in some cases caused a fit of convulsions to start or to intensify. Periods of convulsions were replaced by a state of total adynamia. In some cases the interchange of these phases was quite rhythmic in character over a definite interval of time.

The pathologoanatomical picture of the lesion may be summarized as a moderate decrease in the size of the thymus and a more marked decrease in that of the spleen, coupled with severe vascular disorders. Severe hemorrhages were also seen in the lungs but were completely absent in the brain.

SUMMARY

The effect of large x-rays doses upon young rats, aged from 25 to 30 days, was compared with that provoked in 3–4-month-old animals (sexually mature). Age was an important factor predetermining the extent of radiation injury. The role of this factor is manifested in the wide range of doses–from 10,000 to 50,000 r. Immature rats are more vulnerable than the sexually mature animals. The development of the so-called acutest form of radiation sickness deserves special attention. A shock-like condition, paralyses, convulsions and other symptoms occurred earlier and were more severe in the sexually immature rats. The age differences diminish with the increase of the dose. The threshold dose is probably about 50,000 r.

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RELATIONSHIP BETWEEN CHANGES IN MITOTIC ACTIVITY OF THE CORNEAL EPITHELIUM AND BODY WEIGHT OF FASTING ANIMALS

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Although some research has been done on the role of energy metabolism in the regulation of the mitotic activity of the body tissues [5, 6] much remains to be explained. In the study of this problem it is advisable to carry out experiments during fasting, when the energy reserves of the body are considerably depleted.

The course of various physiological processes during starvation has been studied in adequate detail. We know, in particular, that in young animals whose weight is small, the sharp decrease in the gas exchange (on account of a decrease in the amount of oxidizable material in the body) takes place much sooner than in adult animals, in which this decrease is preceded by a long (7-8 days) period of stable gas exchange [4]. These differences in the time of the decrease in gas exchange in small and large animals during fasting are the result of differences in the expenditure of energy: the expenditure of energy per unit body weight and surface area is much greater in young animals than in adults [4].

Studies of the mitotic activity of the epidermis, the intestinal epithelium and the liver of certain mammals [7, 8, 9] have shown that the number of dividing cells falls during starvation. The question of a connection between the mitotic activity and the character of the processes taking place in the starving animal, however, has not yet been investigated.

In a previous communication [2] we showed that the decrease in mitotic activity taking place on the third day of starvation in rats of small body weight (60 g) was preceded by a considerable increase in the number of dividing cells in the corneal epithelium. In mice, which are characterized by a higher level of the basal metabolism, the fall took place 24 hours earlier than in rats [3].

The object of the present investigation was to study the changes in mitotic activity, taking into account differences in the expenditure of energy in animals of the same species but of different body weight and age, during total and prolonged fasting.

METHOD

Experiments were carried out on male white rats of different body weight: 1) on young, sexually immature animals weighing 55-60 g (low body weight group); 2) on sexually mature animals weighing 157 g (average body weight group); 3) on old animals weighing 332 g (high body weight group).

*Deceased.

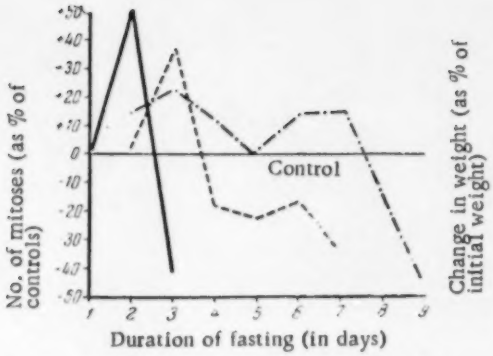

Fig. 1. Changes in the mitotic activity of the corneal epithelium and in the body weight of fasting rats kept at an optimal temperature.

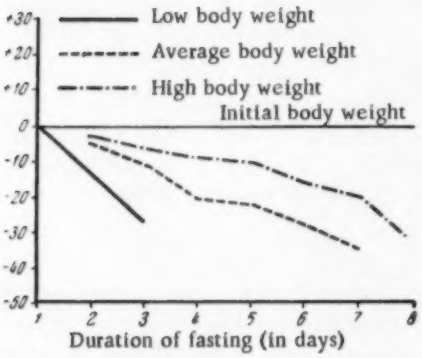

Fig. 2. Changes in body weight and mitotic activity of white rats during fasting

Before the experiment started, the rats were kept under standard conditions for several days. The air temperature in the experimental room varied between 20° and 24°. Control and experimental groups comprised five animals in each variant of the experiment. Fasting in our experiments lasted for one to nine days. The fasting animals received nothing but water. The rats were weighed during the experiments. The control and experimental animals were sacrificed in turn, and always at the same time of day.

In one series of experiments an increase in the intensity of metabolism was brought about in the starving animals. For this purpose the rats were kept at a lowered temperature (16°). The control group was kept at 24-25°.

The mitotic activity was investigated in histological preparations of the corneal epithelium, stained by Carazzi's hematoxylin.

The mitotic index was taken to be the number of divisions taking place on the average in 100 fields of vision, corresponding to 1 mm^2 surface area of the cornea.

RESULTS

1. Starvation of rats of different body weights in optimal temperature conditions.

The weight of the experimental animals and the mitotic indices of the corneal epithelium during fasting changed consistently. The mean figures showing these changes are given in Table 1, and in Figs. 1 and 2. The changes in the mitotic activity of the corneal epithelium and in the body weight of the starving animals are shown graphically. It can be seen that the changes in the mitotic activity were closely related to the changes in body weight observed in the 55-60g rats during three days of starvation. We noted a maximum increase in the number of mitoses in these animals on the second day of starvation (to 51%) and a sharp fall in the number of mitoses at the end of the experiment.

In rats weighing 157g, the fall in body weight was less pronounced and the increase in the number of mitoses observed in these animals on the third day of fasting did not exceed 39%. A fall in mitotic activity was observed only after the fourth day of the experiment. This fall continued to progress until the seventh day inclusive.

In the rats weighing 332g the loss of weight during fasting was least marked. The maximum increase in mitotic activity did not exceed 24%, but a tendency towards an increase in the number of mitoses took place at a much later period (until the seventh day of starvation). After nine days of fasting the mitotic activity also fell in the animals of this weight category.

2. Starvation of rats at a lowered temperature.

The results of these experiments provided a basis for the hypothesis that the intensity of mitotic activity and the degree of depletion of the energy resources of the body are correlated. In order to verify this hypothesis we carried out experiments to increase the expenditure of energy during starvation. For this purpose the fasting and control animals were kept in a room at a lowered temperature (16°). At the same time an experiment was conducted in a room where the temperature was maintained at the optimal level (23-24°). The mean values of the mitotic indices obtained in these experiments are shown in Table 2.

TABLE 1

Changes in the Body Weight and Mitotic Activity of Rats During Fasting

Duration of experiment (in days)	No. of animals	Mitotic index	Control series: change in body wt (as % of original)	Control series: no. of animals	Control series: mitotic index	Experimental series: change in no. of mitoses (as % of controls)	Experimental series: change in body wt (as % of original)	Experimental series: probability of chance difference in mitotic index compared with controls
Low body weight group of rats								
1	10	347	no change	10	346	no change	no change	
2	10	292	+8	10	441	+51	−13.7	P = 0.018
3	10	306	+12	10	181	−41	−27.7	P = 0.027
Average body weight group of rats								
2	10	274	no change	10	279	no change	− 6	
3	10	231	no change	10	321	+39	−12	P = 0.061
4	10	300	+3	10	247	−18	−20	
5	5	284	+2	5	218	−23	−23	
6	5	273	no change	5	230	−16	−28	P = 0.0000
7	5	275	+7	5	182	−34	−35	
High body weight group of rats								
2	10	264	no change	10	301	+14	− 3	
3	10	221	no change	10	275	+24	− 7.5	
4	5	250	+3	5	280	+12	− 9	P = 0.0000
5	5	141	no change	5	139	− 1	−11	
6	10	231	+4	10	265	+14	−16.5	
7	5	218	+5	5	252	+15	−20	
9	5	326	+1	5	177	−46	−29	P = 0.027

TABLE 2

Mitotic Activity of the Corneal Epithelium of White Rats on the Third Day of Fasting at Different Temperatures

Temperature of room	Average body wt. (in g)	Mitotic index: control animals	Mitotic index: experimental animals	Change in mitotic index as % of control	Fall in body wt. as % of initial, after 2 days of experiment
24—25°	200	260∓37,9	315∓19,1	+20	—9
	57	444∓47,9	311∓30,8	—30	—16
16°	200	428∓51,6	276∓11	—36	—22
	57	437	2	in 218 times	—39

[1] The animals were not weighed on the day they were sacrificed.

It can be seen from the figures given in Table 2 that an increase in the basal metabolism of the fasting animals resulting from an increase in the expenditure of energy for the needs of heat production led to a decrease of 36% in the mitotic activity, even in the rats weighing 200 g, although in the rats of the same weight but kept at a temperature of 23-24° the mitotic activity was increased by 20%.

The greatest decrease in mitotic activity (218 times greater than in the controls), accompanied by the greatest loss of weight, was found in the rats weighing 57 g, the energy expenditure per unit body weight of which was much greater than in the adult animals. The experimental results described demonstrate the existence of a relationship between the degree of wasting of the animals during starvation and the original weight, and also a connection between the body weight of the animals and the character of the change in the mitotic activity. The smaller the weight of the animals and the higher, although the shorter in duration, the increase in the mitotic activity at the beginning of fasting, the sharper its fall at the end.

In the majority of cases when the fall in the initial weight was 20% an increase in mitotic activity was observed. With a further decrease in weight, the number of dividing cells also diminished.

In the largest animals, distinguished by the smallest loss of body weight, the mitotic activity was increased until the seventh day of starvation inclusive. Only after nine days of starvation did a significant increase take place in the number of mitoses.

When our results are compared with those in the literature, support is obtained for the claim that the mitotic activity persists during starvation. Hooper and Blair [8], for example, who investigated the mitotic activity of the epithelium of the small intestine of fasting rats, observed a slight fall in the number of dividing cells only on the fifth day of the experiment. Similar results were obtained by A. N. Zorin [1].

Our experiments give evidence of the high proliferative power of the corneal epithelium even during starvation, an indication that synthetic processes continue to take place in this structure at a high level. A decrease in the mitotic activity took place only in the stage of severe emaciation of the animal. We consider that the quicker and more acute fall in the mitotic activity of the smaller rats if related to their more intensive basal metabolism and, consequently, to the more rapid expenditure of energy-producing material, necessary for the maintenance of various physiological processes, including cell proliferation. The latter is confirmed by the experiment in which starving rats of low body weight were kept for three days at a lowered temperature. This led to the rapid expenditure and depletion of the energy resources of the animals for the production of heat, and to the almost complete disappearance of mitoses in the corneal epithelium. In the animals which had fasted for three days, but which were kept at a higher temperature, no such sharp fall in mitotic activity took place anywhere.

We have previously found in white mice [3] that compensation for the expenditure of energy by carbohydrates alone enables the the mitotic activity of the corneal epithelium to be maintained at a normal level until the fourth day of fasting inclusive. This suggests that the energy resources of the body play a most essential part in cell proliferation.

SUMMARY

Fasting for 24 hours did not change the number of dividing cells of the corneal epithelium in rats with the average weight of 55-60 g, whereas 48-hour fasting was accompanied by its intensification; mitotic activity was found to be considerably reduced after 72 hr of the experiment. After an initial rise, observed for up to 72 hr of fasting, the mitotic activity was found to decrease on the 4th, 5th, 6th, and 7th days in rats with an average weight of 157 g. The maximal reduction of mitotic activity (34%) and of the body weight (35%) was revealed on the 7th experimental day.

In rats with average weight of 332 g the mitotic activity increased by 13% on the average (as compared with controls) during fasting from 2 to 7 days. Reduction in the number of dividing cells in this weight group was noted after 9 days of fasting. The most rapid reduction of the weight and mitotic activity occurred in rats with the least weight. In medium-weight and high-weight rats such changes took place later. Intensification of metabolism in the fasting animals, connected with the increased energy expenditure for replenishment of thermal requirements (with the animals kept at 16°C) produced maximal reduction of mitotic activity and the greatest loss of weight in the smallest rats.

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PENICILLIN PERMEABILITY OF THE PLACENTAL BARRIER AT VARIOUS STAGES OF PREGNANCY

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Despite some progress made in the field of study of the fundamental properties of the placenta, many problems concerned with the functions of this organ have so far received inadequate attention. A problem worthy of study is the passage of drugs, introduced into the mother for prophylactic or therapeutic purposes, through the placenta.

Many authors have demonstrated the passage of drugs through the placenta in animals and man: streptocide [4], labeled caffeine [5], antipyrin [1], streptomycin [3,11,12], erythromycin [9], vitamins [6,10] and so on.

In order to discover the action of drugs on the development of the intrauterine fetus, however, it is important to determine the degree of permeability of the placental barrier at various stages of pregnancy. The study of the permeability of the placenta to drugs is of great interest in connection with the real risk of intrauterine involvement of the fetus in certain diseases of the mother.

Antibiotics are used at the present time for the prevention and treatment of many diseases in pregnant women. Penicillin is widely used for these purposes. Several workers have concerned themselves with the study of the permeability of the placenta to this particular antibiotic [2,7,8]. They have investigated the penicillin permeability of the placenta during labor or immediately before.

The extension of our knowledge of the degree of permeability of the placenta to penicillin, administered to the mother at various stages of pregnancy, would be of help in the study of problems concerned with the antenatal care of the fetus.

Our object was to make an experimental study of the permeability of the placenta to penicillin at the principal stages of embryonic development.

METHOD

For the experiments we used primiparous white rats, bearing in mind that this species of laboratory animal is often chosen for experimental research. Moreover, the placenta of this species of animal, like the human placenta, has a hemochorial type of structure. To obtain pregnant animals, females were caged with males for 24 hours (in a ratio of one male to 5-6 females). The rat was considered to be pregnant from the moment that spermatozoa were found in the vaginal smear. The initial body weight of the rats on the first day of pregnancy was 170-200 g.

Penicillin Concentration in the Blood of Pregnant Rats and Fetal Tissues after Injection of the Antibiotic at Various Stages of Pregnancy (in units/ml)

Day of pregnancy	Material investigated	Time after injection of penicillin					
		30 min		2 hr		3 hr	
		no. of animals	concentration of penicillin	no. of animals	concentration of penicillin	no. of animals	concentration of penicillin
11th	Blood of pregnant rats	8	61.44–15.36 (mean 23.0)	6	0.96–0.24 (mean 0.48)	7	0.48–0.12 (mean 0.26)
	Fetal tissues	8	0.24 – 0.03 (mean 0.08)	6	0.12–0.03 (mean 0.07)	7	0.12–0.03 (mean 0.06)
16th	Blood of pregnant rats	6	30.72–15.36 (mean 27.7)	7	0.96–0.24 (mean 0.4)	7	0.24
	Fetal tissues	6	0.12–0.03 (mean 0.06)	7	0.12–0.03 (mean 0.07)	7	0.12–0.03 (mean 0.06)
20th	Blood of pregnant rats	10	30.72–15.36 (mean 26.9)	5	0.48–0.24 (mean 0.33)	6	0.24–0.06 (mean 0.23)
	Fetal tissues	10	0.96–0.48 (mean 0.67)	5	0.96–0.24 (mean 0.64)	6	0.96–0.24 (mean 0.62)

The permeability of the placenta was investigated on the 11th, 16th, and 20th days of pregnancy, and correlated with the developmental and structural features of the placenta in rats at these times.

Penicillin was injected in a dose of 20,000 units/kg body weight. The calcium salt of penicillin was injected intramuscularly into the right hindlimb.

The permeability of the placental barrier was studied by the comparative analysis of the penicillin content of the maternal and fetal tissues by means of the serial dilution micromethod of Z. V. Ermol'eva and E. A. Ved'mina [3]. This method is sufficiently sensitive and can be used to determine the penicillin concentration in the blood with an accuracy of 0.03 units/ml of serum. The test organism was No. 209 Oxford strain of Staphylococcus aureus, and the indicator of growth of the microorganisms was Andrade's reagent. All the experimental animals studied were kept in identical conditions, favoring a normal course of pregnancy.

The experimental rats were divided into three groups corresponding to the days of pregnancy. The animals of the first group (21 rats) received penicillin injections on the 11th day of pregnancy, those of the second group (20 rats) on the 16th day, and those of the third group (21 rats) on the 20th day of pregnancy.

We know from the literature [2] that the penicillin concentration after intramuscular injection reaches a maximum 30 min after injection of the drug, stays at a high level for one hour, gradually falls to the second hour and has almost completely disappeared three hours after injection. Taking these findings as a guide we investigated the penicillin concentration in the blood of the rats and in the tissue juices of the fetuses 30 min and 2 and 3 hr after the drug was injected into the pregnant animals. Blood was obtained from the carotid arteries of the pregnant animals by decapitation. Next, with strict observance of the rules of asepsis, the abdomen was opened and the uterus with the contained fetuses extracted. From 5 to 12 fetuses were taken for investigation, depending on the stage of pregnancy. The fetuses (in the early stages of pregnancy, embryos) were freed from their bed in the uterus, separated from the membranes, dried with filter paper, weighed and ground in a mortar by means of a quartz pestle, after which they were treated with a phosphate buffer (pH 7.2-7.4). The mixture thus obtained was centrifuged and the penicillin concentration in the centrifugate was determined. The penicillin concentrations in the blood of the pregnant rat and in the fetal tissues were expressed in units/ml of the biological fluid tested. The results obtained are shown in the table.

RESULTS

After intramuscular injection of the same dose of penicillin (5000 units) into the animals on the 11th, 16th, and 20th days of pregnancy, variations in the concentration of the antibiotic between different limits were observed. For instance, when determined 30 minutes after injection, the penicillin concentration varied between 61.44 and 15.36 units/ml, falling sharply in the second hour of the investigation to 0.96-0.24 units/ml. After 3 hr the concentration was 0.48-0.12 units/ml. In view of the regularity of the pattern of fall of the penicillin concentration in the blood of the pregnant rats, it may be postulated that to maintain the blood concentration of the antibiotic in pregnant animals, it should be administered at intervals of 3 hours.

A different pattern was observed when the penicillin concentration in the tissues of the embryo was investigated.

Thirty minutes after the intramuscular injection of penicillin into the animals of the second group (11th day of pregnancy), the concentration of the antibiotic in the tissues of the embryos averaged 0.07 units/ml, and remained at approximately this level for 2 and 3 hr.

After the intramuscular injection of penicillin to animals on the 16th day of pregnancy, the concentration of the antibiotic in the fetal tissues was roughly the same as in the embryos of the first group of animals (11th day of pregnancy); when investigated after 30 min it was 0.08 units/ml, and it remained at this level after 2 and 3 hr.

It was thus shown that penicillin can penetrate into the tissues of the embryo even in the early stages of embryogenesis (11th and 16th days), but that its concentration in the embryo was far less than in the maternal blood. The penicillin concentration in the tissues of the embryo was maintained at a constant level longer than in the maternal blood. This is evidently due to the more rapid excretion of penicillin from the mother.

After injection of penicillin into animals on the 20th day of pregnancy it was found that its concentration in the fetal tissues varied between 0.67 and 0.62 units/ml when tested after 30 min. During the next 2-3 hr after injection of the antibiotic, its concentration remained essentially unchanged (0.67-0.62 units/ml).

SUMMARY

Permeability of the placental barrier to penicillin was studied on pregnant rats on the 11th, 16th, and 20th days of pregnancy. The penicillin concentration was compared in the mother's blood and the fetal tissue fluids at various stages of pregnancy 30 min, 2 and 3 hr after its intramuscular administration in a dose of 20,000 units/kg body weight. The penicillin concentration was examined by Ermol'eva and Ved'mina's serial dilution micromethod. The placenta of white rats is permeable to penicillin on the 11th, 16th, and 20th days of pregnancy; it is more permeable on the 20th day than on the 11th and 16th days. The concentration of antibiotic in the mother's blood is higher than in the fetal tissues. A reduction of the blood penicillin level occurs in pregnant rats much more rapidly than in the fetus.

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CHANGES IN THE SYNAPTIC ENDINGS ON NERVE CELLS AND ON THE CAPILLARY WALLS OF THE SPINAL CORD IN DOGS WITH ACUTE RADIATION SICKNESS

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In recent years the physiological and clinical observations indicating the high sensitivity of the nervous system to the influence of ionizing radiation have been augmented by morphological data.

Many facts demonstrating the injury to the nerve cells of various divisions of the brain, the spinal and sympathetic ganglia, the receptors and nerve fibers have been presented by V. V. Portugalov [13] and co-workers.

A. D. Zurabashvili and B. R. Naneishvili [8] studied the stage of certain divisions of the brain and spinal cord of irradiated dogs and monkeys and concluded that the central nervous system is very sensitive to x rays. They found hyperimpregnation of the synaptic structures, which they called "irritation."

By investigating the spinal cord of rats irradiated with a dose of 450-600 r, we [11] found a series of reactive changes (hypertrophy, destruction) of the axo-somatic, axo-dendritic and axo-vasal (to use Dolgo-Saburov's terminology [1-7]) synaptic structures. Intact synaptic formations were also found at the same time, a feature which depended, in our opinion, on their initial functional state, and was an expression of the plastic properties of the nervous system.

The widespread involvement of the nervous system (the brain and spinal cord, the spinal ganglia) in the pathological process of radiation sickness was reported by V. P. Kurkovskii [9], who singled out in particular the vascular lesions (hemorrhages, diapedesis).

The injurious action of whole-body and local irradiation on nerve tissue has also been shown by several foreigh workers (Hicks and Montgomery [19], Arnold, et al., [16], McLaurin and Bailey [20], Bering and Bailey [17]).

The changes in the synaptic structures on the bodies and processes of nerve cells of irradiated animals, however, have been studied only by few workers [8, 11].

In the present investigation we studied the changes in the axo-somatic, axo-dendritic and, in particular, the axo-vasal synaptic endings in the spinal cord of dogs suffering from acute radiation sickness.

*Deceased.

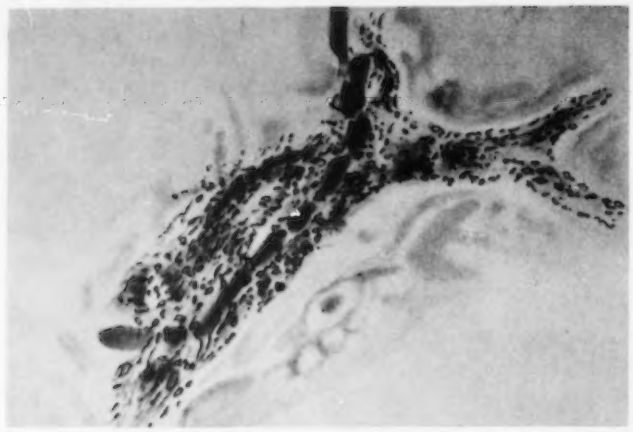

Fig. 1. Lumbar enlargement of the spinal cord. Synaptic structures on the body and processes of the nerve cell and on the walls of a capillary. Impregnated by the Golgi-Deinek method. Immersion.

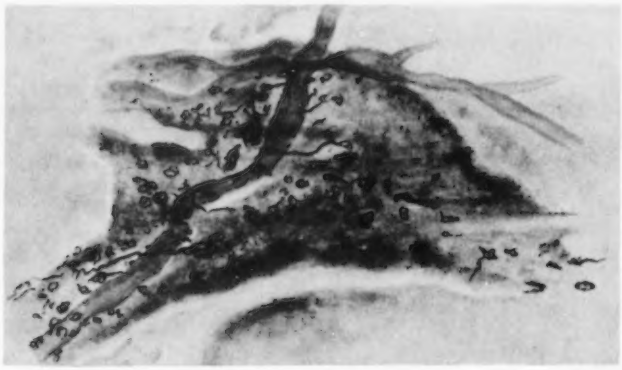

Fig. 2. Cervical enlargement of the spinal cord. Hypertrophic synaptic structures on the body and processes of the nerve cell and on the capillary walls. Impregnation by the Golgi-Deinek method. Immersion.

METHOD

The material used in the investigation consisted of the spinal cord of five adult dogs subjected to irradiation in a dose of 450 r (185 kv, 15 ma, filter 0.5 mm Cu, skin-focus distance 120 cm). The state of acute radiation sickness was diagnosed by blood counts and clinicophysiological observations. The animals died on the 9th-11th day after irradiation, i.e., at the height of the disease.

Fragments of spinal cord were taken from the cervical and lumbar enlargements and also from the thoracic segment, and were treated by the Golgi-Deinek method, and embedded in celloidin. Sections were cut to a thickness of 22-24μ in three mutually perpendicular directions, and the capillaries were counterstained with acid fuchsin.

RESULTS

The grey matter in the region of the cervical and lumbar enlargements is known to be the richest in nerve cells; here there is correspondingly a more intensive development of the network of blood vessels, in close topo-

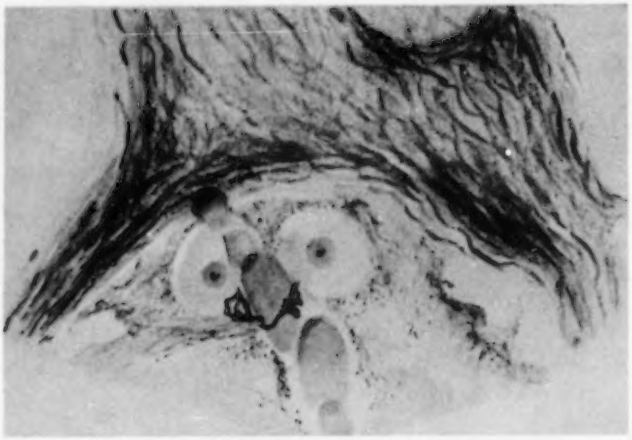

Fig. 3. Lumbar enlargement of the spinal cord. Hypertrophy and commencing destruction of the synaptic ending in the wall of a capillary. Impregnation by the Golgi-Deinek method. Immersion.

graphical relationship to the nerve cells [10]. In the plane of the transverse section of the grey matter a larger number of vessels is to be found within the limits of the motor nuclei of the anterior horns. We therefore concentrated our attention in particular on the study of the reactive changes in the synaptic structures, especially the axo-vasal, in these divisions of the grey matter of the spinal cord.

The reaction of the synaptic structures on the nerve cells and surrounding capillaries to ionizing radiation takes various forms: an increase in their argyrophilia, changes in their shape (deformation), hypertrophy, signs of destruction and disintegration. Synaptic structures began to show themselves in numbers not observed in normal conditions. Synaptic loops or, more rarely, boutons, sometimes supplied with small afferent fibers, were literally strewn over the body of the nerve cell and its process, and were distributed in the walls of the capillaries surrounding the neuron. These capillaries bore the most varied topographical relationships to the nerve cells, sometimes penetrating their body (Fig. 1) or surrounding the root of a dendrite, forming a wide plate or lamella. Another indication of the increased argyrophilia was the abundance of visible presynaptic fibers, often convoluted and with varicosities.

It is not yet possible to point to any morphological features distinguishing the synaptic structures distributed on the nerve cells from the analogous structures on the capillary walls. This fact may be interpreted as a sign of the common origin of these contact apparatuses, which is also suggested by the pattern of division of the afferent nerve fiber into two branches, terminating separately on the neuron and the capillary wall [7], i.e., the multiplication of the nerve conductors. Loops are also found, joined together in dumb-bell fashion.

In the dogs suffering from acute radiation sickness deformed synaptic loops were very often found; distinguished by their polymorphism (round, oval, rhomboidal, triangular, quadrilateral, with irregular outlines and so on). These changes affected both axo-somatic, axo-dendritic and axo-vasal synaptic structures (Fig. 2).

Another type of reaction is hypertrophy of the contact apparatuses. In these cases a sharp increase in the diameter of the loops could be observed (from 2 to 10 times) over their original size, their outline became thickened and the loops themselves more widely separated. This was usually associated with a reduction in the total number of synaptic structures on the neuron. Deformation and hypertrophy also affected the axo-vasal synaptic structures (Fig. 2); thickened and convoluted afferent fibers were often in this situation.

The destruction which affected part of the contact apparatuses was shown by loss of continuity of the loops, the appearance of vascuoles in the boutons, and by disintegration of the terminal structures (Fig. 3). The final stage of this process was the accumulation of granules, forming figures bearing a distant resemblance to loops with afferent fibers.

The findings described, in our opinion, reflect different stages of the reactive condition of the synaptic structures in response to the action of an extremely inadequate stimulus, and are not the expression of the "vital process of development and death of the ending," first described by Weber in 1945, and recently by Charvat [18].

The deformation and destruction of the contact apparatuses led to some decrease in their total number. Nevertheless, the increase in the number of synaptic structures which we observed as a result of the enhanced argyrophilia cannot be regarded as merely the more complete manifestation of already existing structures. In the present state of our knowledge it may be postulated that under the influence of ionizing radiation excessive growth of synaptic structures—i.e., hyperplasia—may take place. Grounds for this hypothesis are provided by the fact that new processes and new branches and endings develop in the course of activity of the neuron. This may also occur in radiation sickness, which leads, as we know, not only to degeneration of the various structures, but also to their possible regeneration [12].

Side by side with synaptic structures on the neurons and capillaries exhibiting the described changes, unchanged structures were often encountered. This is not surprising, for the radiosensitivity of both the nervous and the contact apparatuses of communication depends on their functional state, which may vary.

The ability of the irradiated animal (almost to the time of death from acute radiation sickness) to perform locomotor and other activities involving the participation of the nerve cells of the spinal cord suggests that the compensatory mechanisms of the nervous system are intact. The plasticity of these mechanisms may evidently be dependent on the presence of intact neurons, with synaptic structures on them and on the capillaries, among the damaged neurons.

It may be concluded from these investigations that reactive changes are observed in the axo-somatic, axo-dendritic and axo-vasal structures in the spinal cord of dogs suffering from acute radiation sickness.

These reactive changes appear in various forms: the affinity of the synaptic structures for silver salts is increased (argyrophilia), and deformation, hypertrophy and even destruction of part of the synaptic structures take place. Some synaptic structures, remaining morphologically intact, evidently retain their functional powers and enable the compensatory properties of the nervous system to be brought into play in irradiated animals.

SUMMARY

In dogs suffering from acute radiation sickness (the irradiation dose being 450 r) the reaction of synaptic formations in cells and capillary walls consists of two phenomena: a) increased affinity of the synaptic structure for silver (increased argyrophilia); they are detected in numbers, greater than under normal conditions; b) deformation, hypertrophy and destruction of synaptic formations.

The picture of the reactively changed synaptic endings is variable. The presence of intact structures on neurons and capillaries points to the plasticity of the nervous system providing an optimal level of vital processes under given conditions of existence.

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THE ACTION OF ULTRASOUND ON PERIPHERAL NERVE FIBERS AND NERVE ENDINGS

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During the last two decades many reports have been published on the therapeutic use of ultrasound. Ultrasonic therapy has been used particularly in neurological diseases. The influence of ultrasound on the peripheral nervous system has, however, been neglected. Most research has been devoted to the study of the action of high-intensity ultrasound, which has a destructive action on nerve tissue.

Only isolated communications have appeared dealing with the influence of therapeutic doses of ultrasound on the peripheral nervous system. R. Pohlman [2] in particular states that therapeutic doses ultrasound have a very weak action on healthy nerves and nerve endings. N. P. Krylov and V. I. Rokityanskii [1] draw attention to the high sensitivity of the nerve endings to the action of ultrasound, basing this conclusion on the development of pain in animals in the area so treated.

We found no reference in the Soviet literature specifically concerned with the morphological changes in the peripheral nerve fibers and endings resulting from the action of therapeutic doses of ultrasound, and we therefore decided to study this problem.

METHOD

We carried out experiments on 20 guinea pigs (see table).

The first series of experiments was conducted on 14 animals which were irradiated with ultrasound of an intensity of 0.5 W/cm^2 and frequency 1625 kc with pulsed operation of the generator; in six cases irradiation lasted 3 minutes, in four cases—5 min and in four more—10 min.

In the second series of experiments on six guinea pigs ultrasound was applied with an intensity of 1 W/cm^2 and frequency of 1625 kc with continuous operation of the generator. Irradiation lasted 3 min.

In all the experiments contact between the vibrator and the skin surface was made through vaseline oil. The lateral surface of the right thigh, from which the hair was removed, was irradiated. During irradiation the head of the vibrator was slowly moved about through very short distances, so that the central area of skin did not escape the action of the ultrasound.

Under the action of ultrasound the animals always appeared restless, giving frequent, repeated jerky movements. After irradiation, only in one case did we observe a state of paresis of the corresponding limb of the animal, passing off after two hours, and in the remaining cases the animals behaved normally.

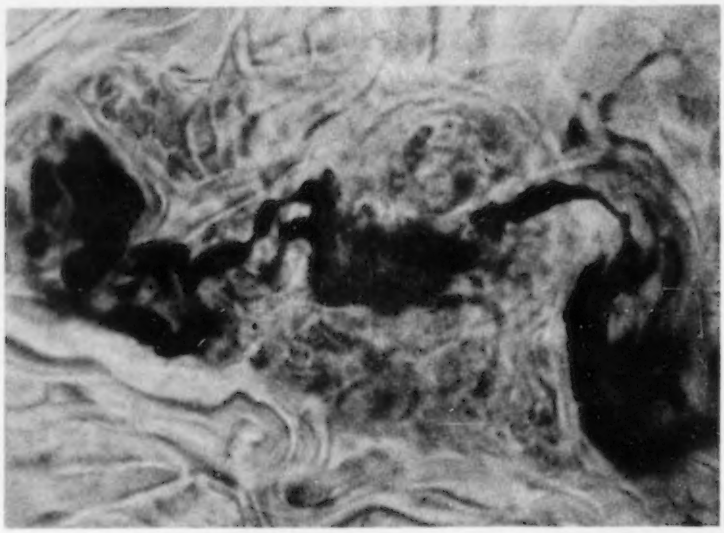

Fig. 1. Skin 3 days after irradiation with continuous ultrasound. Nerve fibers undergoing swelling and fragmentation can be seen. Silver impregnation by the Bielschowsky-Gros method, with subsequent gilding. Photomicrograph. Objective 40 ×, ocular 10 ×.

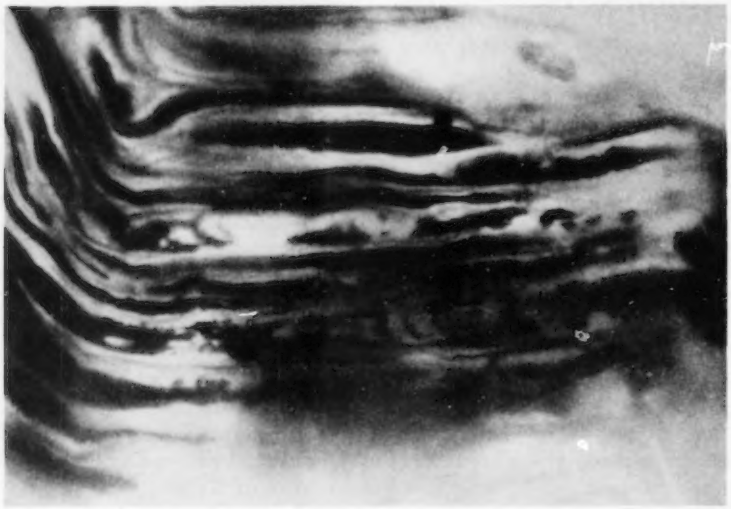

Fig. 2. Sciatic nerve 3 days after irradiation with continuous ultrasound. Irregularly thickened axis cylinders with signs of dyschromia are seen side by side with axis cylinders undergoing vacuolation and fragmentation. Impregnation by the Bielschowsky-Gros method. Photomicrograph. Objective 40 ×, ocular 10 ×.

Specimens for investigation were taken 2 hours and 1, 2, and 3 days after irradiation. In order to study the action of ultrasound on the peripheral nerve fibers and endings, the skin, underlying muscle tissue and a portion of the sciatic nerve were removed from the irradiated area. As controls we used corresponding material from the unirradiated left thigh. The specimens were fixed in 10% neutral formalin. Microscopic preparations were impregnated with silver by the Bielschowsky-Gros method, often followed by gilding and staining with hematoxylin-eosin.

Expe imental Conditions

Series of experiments	Intensity of ultrasound (in W/cm²)	Frequency of ultrasound (in kc)	Mode of operation of generator	Time of irradiation (in min)	No. of experimental animals	Time from moment of irradiation to taking of specimens
First	0.5	1625	Pulsed	3	2	2 hr
					2	1 day
					2	3 days
				5	2	2 hr
					2	3 days
				10	2	2 hr
					2	3 days
Second	1	1625	Continuous	3	2	2 hr
					2	2 days
					2	3 days

RESULTS

Microscopic examination of the specimens obtained in the first series of experiments, when the action of ultrasound lasted 3 min, revealed no changes in the nerve fibers and nerve endings in the skin, muscle tissue and sciatic nerve. In the skin of the other guinea pigs, irradiated for 5 and, in particular, for 10 min, side by side with unchanged nerve fibers could be seen other fibers whose axis cylinders appeared swollen and contained varicosities; the latter often gave the axis cylinders the appearance of a string of beads. The swellings were usually small in size, oval or round in shape, and homogeneous or loosely woven in texture.

Varicose axis cylinders were characteristically found in nerve fibers in the early stages, 2 hr after irradiation with ultrasound, and in far smaller numbers after one and three days; this indicates the reversible character of the changes. The free nerve endings in the skin and the motor end-plates in the muscle fibers remained visibly unchanged. Axis cylinders with indented, uneven outlines and signs of loosening of the structure and dyschromia were seen from time to time in the sciatic nerve after irradiation for 10 min.

Disintegration of the axis cylinders into fragments was not observed in the first series of experiments. It was found incidentally that, 2 hr after irradiation with ultrasound for 10 min, the lumen of the cutaneous vessels was greatly dilated and fully distended with blood cells. On the third day after this degree of irradiation, dilatation of the lumen of these vessels was observed to be less marked and not present in every case. Our findings thus agree in this respect with those of N. P. Krylov and V. I. Rokityanskii [1], who reported hyperemia of the tissues after a single irradiation of animals.

In the second series of experiments, in which constant operation of the generator was used, the changes observed in the first series were present but were more pronounced in character. In two cases in the second series of experiments, moreover, side by side with intact nerve fibers in the skin and muscle tissue we observed a large number of fibers with signs of vacuolation and disintegration into fragments (Fig. 1).

Some of the motor endings in the muscle fibers were also in a state of granular disintegration. In the sciatic nerve in these cases, on the third day after irradiation, a considerable number of nerve fibers was seen whose axis cylinders had disintegrated into fragments. Nerve fibers undergoing fragmentation mingled with unchanged fibers or with fibers whose axis cylinders were irregularly thickened, with signs of dyschromia. Often the outlines of the axis cylinders were indented and tortuous in appearance (Fig. 2).

It follows from the foregoing account that the morphological changes arising in the tissues as a result of the action of ultrasound are primarily dependent upon the mode of operation of the generator: with pulsed ultrasound the changes are expressed to a much smaller degree than when the generator operates continuously.

SUMMARY

Changes were revealed in the peripheral nerve fibers in the form of reversible irritation phenomena following irradiation of guinea pigs extremities with therapeutic doses of pulsed ultrasound, the intensity being 0.5 W/cm^2 and the frequency 1625 kc for 5 to 10 min. In irradiating for 3 minutes with constant ultrasound, the intensity being 1 W/cm^2 and the frequency – 1625 kc, the changes seen in the nerve, skin and muscles were not only reversible but also degenerative.

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THE INSERTION OF ELECTRODES INTO THE HYPOTHALAMIC REGION IN DOGS

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For tapping action potentials and electrical stimulation of the subcortical structures of the brain, a number of methods of insertion of electrodes has recently been suggested. The large number of such methods suggests that none is free from defects. For example, the insertion of electrodes from above [2, 3] is accompanied by trauma to the overlying divisions of the brain; the operation itself, moreover, is technically complicated, for it necessitates operating in the danger zone of the sagittal sinus.

The method suggested by P. G. Bogach and A. F. Kosenko [1] is unsatisfactory for, when the plexiglass shoe containing the electrodes is applied some stretching of the pedicle of the hypophysis is unavoidable, and the presence of a foreign body of considerable size cannot but affect the function of the hypothalamus. The possibilities of G. Ya. Khvoles's [4] method are limited, because only one electrode is inserted for a unipolar lead.

We have adopted a new method based on the use of the plastic AST-T, which possesses properties that make it a valuable material for various types of experimental work. Plastic AST-T solidifies at 30-35°, is durable, a good insulator, causes no particular reaction in surrounding tissues after insertion and, as bacteriological investigations have shown, in its recently prepared form it is sterile.

The operation is carried out under hexobarbital or intratracheal ether anesthesia. The dog is bound to the table lying on its back. A pillow is placed under its neck so that blood does not flow into the trachea. The upper jaw of the animal is bound to the table, and the lower jaw is drawn apart by the assistant; the latter also draws the tongue to one side by means of tongue-holding forceps. The soft palate is divided by a median incision 3-4 cm long; the wound edges are separated by retractors. The mucous membrane covering the palatine bone is incised crosswise and lifted together with the periosteum by means of a raspatory; the suture between the palatine and the sphenoid bones. To approach the anterior hypothalamic region at the level of this suture, a hole 5-7 mm in diameter is bored vertically downward with a spherical drill. The hemorrhage developing during drilling may usually be easily controlled by firmly packing the hole for 30-60 sec. When approaching the dura mater care must be taken that it is not damaged by the drill. The hole itself should be slightly enlarged inwardly, making it barrel-shaped or the shape of an inverted cone. When a sufficiently large area of the dura mater is exposed, the orifice in the bone is irrigated with furacilin, after which it is ready for insertion of the electrodes. It is best to take the lead ends of the electrodes first through the nasal passages with a wire loop. The dura mater is punctured with the sterile ends of the electrodes, which are then at once introduced to the required depth. The cerebrospinal fluid which leaks out is mopped up with gauze strips. To obtain good fixation it is essential to dry out the hold and surrounding bone as well as possible.

The plastic is mixed in proportion of powder to monomer of 2:1 to 2:1.5. The mixture swells to the consistency of sour cream, and it is then poured from a small spoon into the hold (in slight excess). Further solidifi-

cation of the plastic takes place in the hole; when it is becoming solid firm pressure must be applied to it in some way or other and the pressure must not be relaxed until solidification is complete. By means of a trocar, the ends of the electrodes are brought out on the dorsum of the nose. The soft palate is repaired with 2-3 sutures. Insertion of the electrodes into the posterior hypothalamic region is carried out almost in the same way, the only difference being that the hole is drilled 6-10 mm posterior to the suture.

We used electrodes of nichrome wire (diameter 0.2 mm) covered with lacquer over their whole extent except the tip. The nichrome was soldered to a fine multicore copper wire with vinyl chloride insulation. The soldered junction was coated with insulating lacquer and the vinyl chloride insulation of the wire drawn over it. Coming up against the dura mater, the vinyl chloride restricted the penetration of the electrodes. Naturally electrodes of any other construction may be used.

The method described is comparatively simple and enables electrodes to be introduced into any part of the hypothalamic region with minimum trauma to the brain tissue. The fixation of the electrodes is so firm that 30-40 days later, when the dogs were sacrificed at the end of the experiment, the plastic seal with the electrodes could only be removed by breaking the surrounding bone.

SUMMARY

The author suggests a new method of implantation of electrodes through a trephine opening in the hard palate. Fixation of electrodes in the bone opening is effected with the aid of quick-setting plastic AST-T. The method permits the introduction of several pairs of electrodes to the required depth. The external ends of the electrodes are located on the bridge of the nose.

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AN ELECTROCHEMICAL METHOD OF RECORDING THE PATTERN OF OXYGEN METABOLISM IN THE TISSUES IN VIVO

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Insufficient attention is paid in the literature to methods of measurement of the oxygen metabolism in the organs and tissues of the living animal. The polarographic method is the most suitable for this purpose, using any form of solid polarized cathode. The cathode must make contact with the tissues only through a definite part of its metallic surface of strictly constant area (~1-3 mm^2); the rest of the cathode must be carefully insulated electrically. The material of the cathode must be chosen so that it is fully polarizing and, at the same time, enables reproduction of the measurements. The electrical circuit of the apparatus must be simple. We recommend the use of a modified visual polarograph, in which, in contrast to the usual circuits of polarographs with a solid cathode [1, 3], the four-volt battery, potentiometer, voltmeter and calomel element are absent. The suggested circuit is illustrated in Fig. 1. It consists of two electrodes, a galvanometer G and a shunt R.

The resistance of R must be equal to the external critical resistance of the galvanometer, the necessary sensitivity of which is obtained by means of the rider D.

A similar circuit, but with Au—Zn electrodes, has been used in the construction of the "oxygen lead" for measurement of the dissolved oxygen in the depths of reservoirs [4]. The polarized cathode was gold plate and the anode a zinc plate. The necessity of having an external source of tension and of regulating and controlling it was eliminated, because the zinc, dissolving in the water, maintains a constant potential difference required in the Au—Zn electrode couple to reduce the oxygen on the gold to hydrogen peroxide.

We tested the applicability of this electrode couple to the recording of the oxygen metabolism in the muscle of the rabbit (see Fig. 3). In the recording, difficulty was experienced in insulating the gold electrode. We therefore looked for another electrode couple which would be more readily prepared. The most suitable couple was found to be copper amalgam—iron. As polarized cathode we used ordinary lacquered copper armature wire PEL with a diameter of 0.5-0.9 mm. The insulating coating provided on this wire, when applied by the method used in the factory, usually possesses high mechanical and electrically insulating properties. The tip of the wire, sharpened to a point, is carefully amalgamated with mercury. The second electrode is an ordinary carbon steel needle. Stainless or any other type of steel with a passive surface is unsuitable for this purpose. The surface area of the steel electrode is not of significance: it should be at least 2-3 times as large as the active surface of the amalgamated electrode. Flexible leads with vinyl chloride insulation, soldered to the electrodes, are connected to the shunt of a mirror galvanometer, possessing a sensitivity of not less than 10^{-8} a/mm of the scale and an internal resistance of not less than 1000 ohm.

As shown by a calibration experiment in vitro (Fig. 2), the electrode couple Cu(Hg)—Fe which we selected gives a linear relationship between the strength of the surging depolarization current and the oxygen concentration in the solution. The surging current when working with a fixed cathode in polarography is defined as the

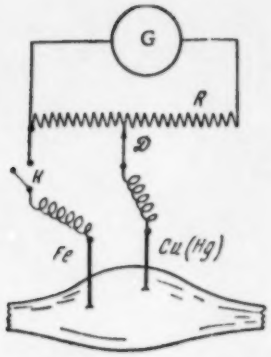

Fig. 1. Electrical circuit of the apparatus.

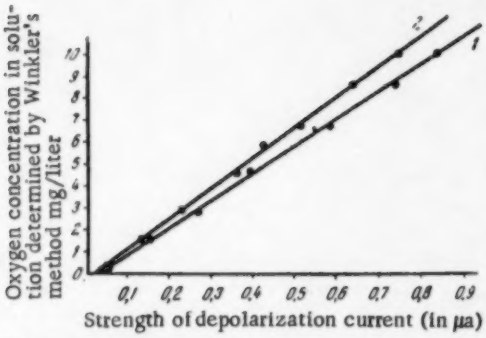
Oxygen concentration in solution determined by Winkler's method mg/liter
Strength of depolarization current (in μa)
0,1 0,2 0,3 0,4 0,5 0,6 0,7 0,8 0,9
1
2

Fig. 2. Calibration chart of an electrode couple copper amalgam–iron. Area of amalgam surface 1.6 mm^2. Ringer's solution, T = 20°. 1) Exposure 30 sec; 2) exposure 60 sec.

current recorded during a short, conventionally chosen and accurately reproducible time of exposure (for example, 30 or 60 sec) after the closure of the circuit, before the current is established and while it still continues to fall [2].

The Au–Zn electrode couple with a gold cathode of equal surface area gives precisely the same calibration curve. Consequently, in accordance with the changes in the strength of the depolarization current it is possible to estimate the changes in the oxygen metabolism in the tissue in which the amalgam electrode is implanted.

We reproduce two experiments on rabbits using a gold (Fig. 3) and amalgam (Fig. 4) electrodes. Both experiments were carried out in the laboratory of the physiology and pathology of respiration and circulation of the blood (Head–Prof. M. E. Marshak) of the Institute of Normal and Pathological Physiology (Director–Active Member AMN SSSR V. N. Chernigovskii). In both cases the gas mixtures of different composition were supplied for respiration into the dissected trachea. The active electrodes–amalgam and gold–were implanted in the thigh muscle, the other electrodes anywhere at a distance not less than 1 cm from the first. The strength of the polarization current was recorded by means of a mirror galvanometer (sensitivity 460 mm/μa, internal resistance 535 ohm, external critical resistance 4200 ohm, t 2.5 sec). The true value of the depolarization current of the electrode was calculated from the ratio between the resistance of the shunts and the galvanometer. At the initial moment of closure of the switch K the galvanometer gives a brisk surge of current, and usually after 2-5 min, in the course of which an approximately stationary process of diffusion of oxygen molecules can be established at the cathode, the light indicator of the galvanometer begins to oscillate slowly near some mean value in accordance with the existing state of the oxygen metabolism in the area of tissue that is being investigated.

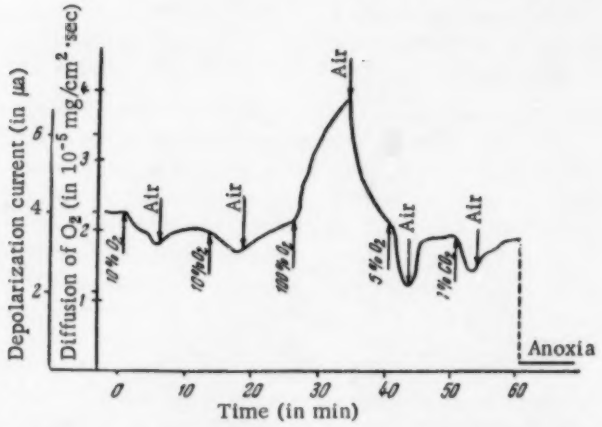

Fig. 3. Changes in oxygen metabolism in the thigh muscle of rabbit no. 1 depending on the composition of the inspired gas mixture. Electrode couple gold–zinc. Area of gold electrode 3 mm^2. Visual recording of mean values of current after every minute (April, 1956).

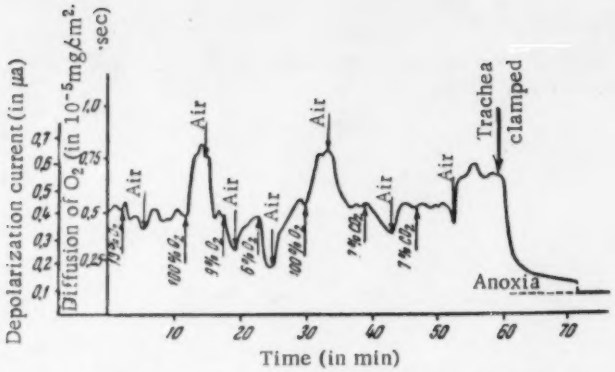

Fig. 4. Changes in oxygen metabolism in the thigh muscle of rabbit no. 2 depending on the composition of the inspired mixture. Electrode couple copper amalgam–iron. Area of amalgam surface 1.3 mm^2. Visual recording of mean values of current after every 15 sec (November, 1956).

As the curves show, besides the small, continuous spontaneous changes in the oxygen metabolism, which are of intrinsic interest, induced variations in metabolism rapidly become established, depending on the composition of the inspired mixture; the inspiration of pure oxygen increases the diffusion of oxygen into the tissues approximately twofold.

The oxygen metabolism in the tissue can be expressed quantitatively in milligrams of oxygen diffusing into the tissues and reduced on 1 cm^2 area of the polarized cathode in 1 sec. This value is proportional to the current density on the surface of the cathode and is calculated by Faraday's second law; the gram-equivalent of oxygen is taken to be 16 g, for under these conditions hydrogen peroxide is formed.

The strength of current remaining after complete anoxia of the tissue should be regarded as the oxygen zero; in the tissues there are other substances which are reduced along with oxygen on the cathode, and which form a small algebraic item which must be subtracted from the galvanometer readings. It is curious that the density of this current was found to be small and equal at the end of the two experiments, although the oxygen metabolism differed very considerably during the experiments.

The polarized cathode, when implanted in a tissue, may imitate the respiring cell; the oxygen utilized by the cathode from the tissue forms an equivalent electrochemical depolarization current, which may be recorded by the galvanometer. The number of molecules of oxygen diffusing to the polarized cathode in unit time, and the equivalent electric current are dependent on many factors: the partial pressure of oxygen in the blood, its coefficient of diffusion, the velocity and volume of the blood flow in the capillaries, the temperature, and the intensity of the oxygen demand by the surrounding cells.

These factors, however, taken as a whole, determine the pattern of the oxygen metabolism in the tissue. The method described is therefore an integrating indicator of the oxygen metabolism in the tissue.

SUMMARY

The author suggests a very simple method for the measurement of oxygen metabolism in animal tissues, which is based on a simplified polarograph system and electrodes with internal galvanic effect. Electrodes, used for the above purpose, consist of a copper cathode with amalgamated active surface, a needle from ordinary carbon steel acting as an anode. The cathode is made from lacquered copper winding PEL, 0.5–0.9 mm in diameter by amalgamating the cone-shaped tip with mercury. The above pair of electrodes, connected through a galvanometer, is inserted into tissues and oxygen metabolism is assessed through galvanometer readings.

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3. A. D. Snezhko, Biofizika 1, 6, 585 (1956).
4. W. Ohle, Die chemische und die electrochemische Bestimmung des molekular gelösten Sauerstoffs der Binnengewässer (Stuttgart, 1953).

THE USE OF ANGIOCARDIOGRAPHY IN PHYSIOLOGICAL EXPERIMENTS

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(Presented by Active Member AMN SSSR, V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 12,
pp. 108-110, December, 1960
Original article submitted November 16, 1959

The method of contrast angiocardiography, now widely used in clinical practice for the diagnosis of congenital and acquired lesions of the heart and great vessels, has received the attention it deserves in experimental research.

A serious obstacle to the large-scale use of the method of serial contrast radiography of the heart and great vessels is that we lack a special apparatus produced commercially. Several accessories have accordingly been described, designed to take serial films and to be used in conjunction with Soviet x-ray apparatus.

One such attachment has been suggested by I. S. Amosov (Department of Roentgenology and Radiology of the S. M. Kirov Military Medical Academy).

This apparatus is designed to operate the x-ray apparatus with a Warren motor (making one revolution per minute), on the shaft of which are mounted two ebonite disks with 60 metal teeth. Rotation of these disks closes contacts for switching on the high voltage and taking the x-ray photograph, after which the motor operates an electromagnet which releases the brake, so that the cassette holder is moved through a distance of 10 cm, as required for the next photograph. We have employed the same principle.

We have designed and constructed an apparatus with the following modifications: firstly, we have replaced the weight-operated traction system for the cassette holder by a simpler and more foolproof spring mechanism. For smoothness and ease of movement of the cassette holder, this is fitted with ball bearings, and metal runners are mounted on the wooden frame. Instead of a cramp and screw for fixing the apparatus to the x-ray table, for greater simplicity and convenience of mounting and dismounting three brackets are provided, which are fixed by means of screws on the table. We have lengthened the frame itself and have made a special cassette, in which two films can be inserted instead of one. We can thus obtain a series of eight roentgenograms (instead of four as in Amosov's apparatus). Subsequently, by lengthening the cassette and inserting three films we have been able to obtain a series of nine roentgenograms (Fig. 1). The size of each roentgenogram is 40 × 10 cm instead of 30×10 cm. This is of considerable importance, for the extra 10 cm enables visualization of not only the heart and great vessels but also the kidneys, ureters and urinary bladder of the experimental animals on the same film. It thus becomes possible to study simultaneously the excretory function of the urinary system.

The animal (rabbit or cat) is fixed in the horizontal position, prone, on a special table. Under local anesthesia (4 ml of 2% novocain solution) the jugular (or another) vein is exposed and opened between ligatures. Into the proximal end of the vein is introduced a sterile vinyl chloride catheter, filled with cardiotrast solution.

*Deceased.

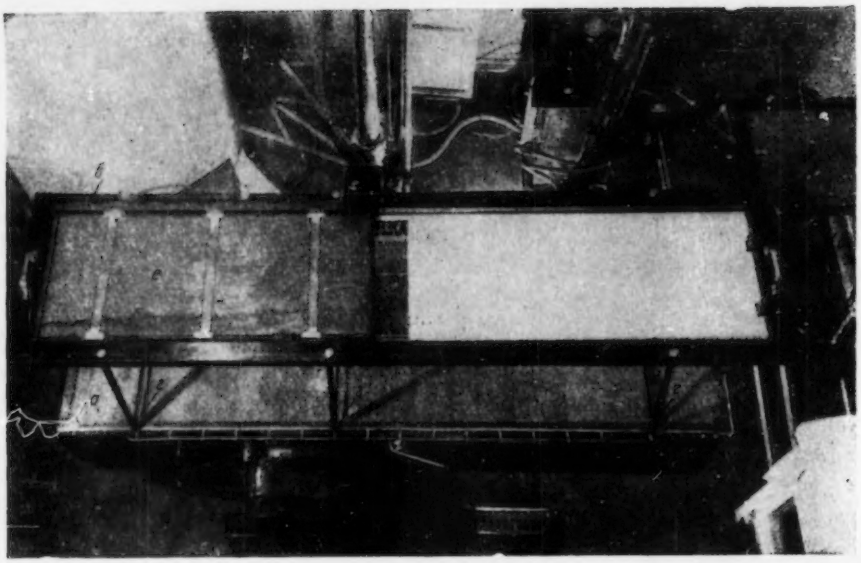

Fig. 1. View of a serial angiocardiography attachment from above. a)Table of URDd 110 k 4 model 1 x-ray apparatus in horizontal position; b) cassette holder; c) spring device for traction; d) brackets; e) fixing screws; f) special cassette.

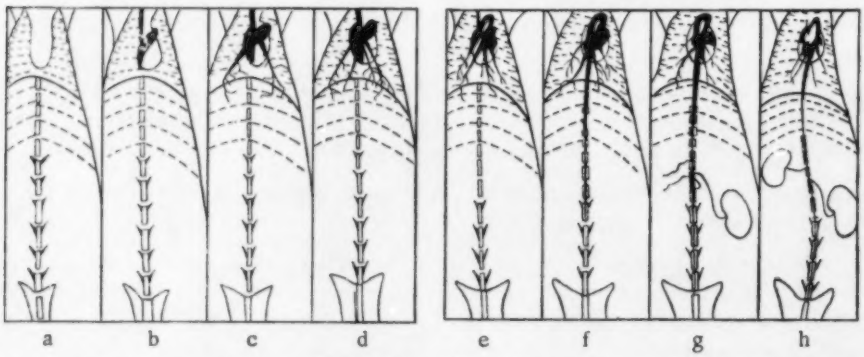

Fig. 2. Serial angiocardiogram of a healthy rabbit. a) Inspection roentgenogram of the chest and abdomen of a healthy rabbit before injection of cardiotrast; b) beginning of injection of cardiotrast, which is entering the right atrium along the superior vena cava from the catheter; c) the same 1.15 sec after the beginning of the injection of cardiotrast. Filling of the right atrium, right ventricle, arch of the pulmonary artery and the pulmonary arteries; d) the same after 2.3 sec. Beginning of filling of pulmonary veins; e) the same after 3.45 sec. The contrast material fills all the chambers of the heart, pulmonary arteries and pulmonary veins. The cardiotrast has reached the arch of the aorta; f) angiocardiogram taken 5 sec after the first film. Cardiotrast can be seen in the pulmonary artery, considerably more in the pulmonary veins, the left atrium and the left ventricle, and there is also filling of the aortic arch and the thoracic and abdominal divisions of the aorta; g) the same after 6 and 15 sec. Traces of contrast material are seen in the pulmonary veins and the left divisions of the heart. The aorta (thoracic and abdominal divisions) are filled throughout their whole extent. The renal arteries can be seen; h) angiocardiogram taken 7.30 sec after the beginning of injection of the contrast material. Cardiotrast is present in the aorta and renal arteries.

For the x-ray examination the animal is placed on the table opposite the cutout portion of the lead shield, after which an inspection roentgenogram is taken. Two seconds later 5 ml of a 35% solution of cardiotrast is injected through the catheter into the vein and the serial angiocardiography is carried out at the same time. After the completion of the experiment the proximal end of the vein is ligated; silk sutures are inserted into the aponeurosis and skin. In the region of the operation wound 50,000 units of penicillin is introduced.

By this method it is possible to determine the time of filling of the chambers of the right heart, the pulmonary veins, pulmonary arteries, the beginning of filling of the left ventricle, the beginning of filling of the arch of the aorta, the descending and abdominal portions of the aorta and the large arteries with contrast material, and also the time of complete evacuation of contrast material from the left ventricle.

It is possible to measure the width of the lumen of the thoracic and abdominal divisions of the aorta, the pulmonary veins and arteries, the individual chambers of the heart and also to determine the size of the heart itself.

By the method of serial angiocardiography it is possible to determine the time of passage of contrast material into the kidneys, of their complete emptying and of the filling of the urinary bladder.

We have studied the roentgenographic picture of the blood flow in 30 healthy rabbits and four cats, which were then tested again after receiving various doses of adrenalin, carbachol, morphine, novocain and other substances. We also carried out observations showing significant changes in the blood flow in anaphylactic shock and also after trauma to the limb of an animal.

By way of example we show a series of angiocardiograms of a healthy rabbit (Fig. 2).

For the more intensive study of some aspects of the circulation of the blood, angiocardiographic investigation should be combined with simultaneous recording of the pulse, the electrocardiogram and the pressure within the chambers of the heart and the vessels. The first results which we have obtained in this direction from combined investigation are promising.

SUMMARY

A simple device, suggested by I. S. Amosov and modified by the authors, made it possible to record 9 serial angiocardiograms, 10 × 40 cm in size, in 8 sec. This proved to be a sufficient number for serial angiocardiography (rabbits, cats) in the assessment of the circulation.

Soviet cardiotrast was used as a contrast medium. The study of these serial angiocardiograms enables the time of filling by the contrast medium of the cardiac cavities, pulmonary veins and arteries, as well as of aorta and large arteries to be determined. The width of the lumen of the great vessels and the individual heart chambers could also be studied. The time when the contrast substance enters and leaves the kidney, as well as the time of filling of the urinary bladder may also be assessed. Various changes occurring in the blood flow as the result of action of different pharmacological substances, in anaphylactic shock or injury of the extremity and distinctly seen on the serial angiocardiograms.

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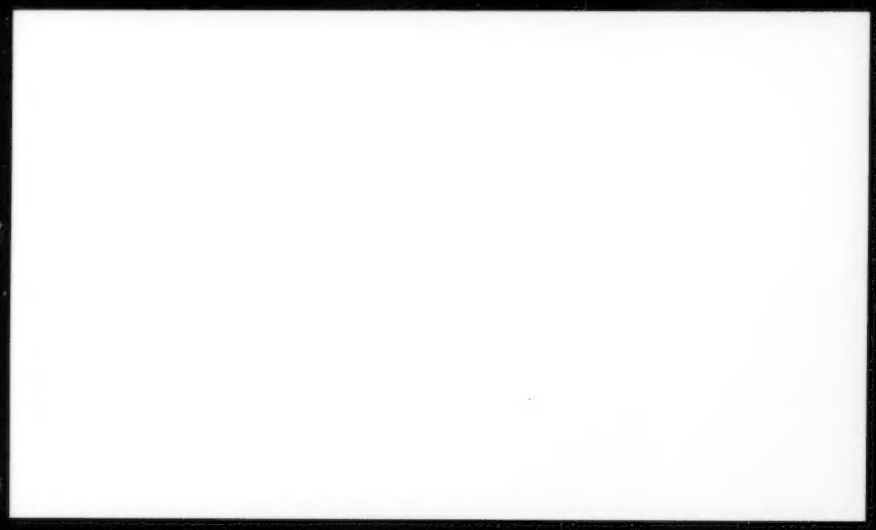

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Sovier Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

PROCEEDINGS OF THE ALL-UNION SCIENTIFIC AND TECHNICAL CONFERENCE ON THE APPLICATION OF RADIOACTIVE ISOTOPES MOSCOW, 1957

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